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Phase I of this program was intended to determine feasibility of using novel absorbable liquid gel-formers (GF) for injectable intravitreal and/or subconjunctival formulations of key drugs for four different indications. And Phase I results do demonstrate the feasibility of preparing injectable controlled release formulations of pilocarpine [PC as a model diagnostic drug used in treating glaucoma], the antiviral ganciclovir [GC for cytomegalovirus (CMV) retinitis], the immunosuppressant cyclosporin A [CP for chronic uveitis], and naproxen [NP as a non-steroidal anti-inflammatory drug]. Results of *in vitro* and *in vivo* release studies, eye examination, and histological evaluation of pertinent tissues indicate that (1) the gel-formers are well suited for intravitreal and subconjunctival injection of 50 to 100 µl of 5% to 10% drug formulation into rabbit eye; (2) administration of those doses does not elicit adverse effects beyond the initial trauma; (3) the continuous flow-cell system designed and employed for monitoring the *in vitro* release profile can be used for accurate comparative study of the *in vitro* controlled release of different drug formulations to timely modulate the formulations for the *in vivo* study; and (5) while the *in vivo* release profiles of the four drugs were generally faster, particularly in the first week than their *in vitro* counterpart, GC continued to release over a study period of three weeks and only 38% of CS was released at two weeks.

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FOREWORD

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TABLE OF CONTENTS

A. INTRODUCTION

This section provides the (1) background to Phase I study and its clinical and scientific significance; (2) technical objectives of Phase I; and (3) related work to Phase I study.

A.1. Background to Phase I Study and its Clinical and Scientific Significance--Treatment of eye conditions has usually been effected using topical ophthalmic drug formulations in either liquid or ointment forms. These are associated with limited efficacy and poor patient compliance for reasons such as instillation difficulty and frequency of dosing. In an early response to this, the Ocusert® controlled delivery system was developed but has had limited success because of difficulty of instillation and likelihood of ejection from the ocular cul-de-sac. This led to sporadic explorations of solid or semi-solid subconjunctival implants for controlled drug delivery. Meanwhile, there has been growing interest in delivering drugs in hard-to-reach parts of the inner eye for treatment of retinal lesions, such as those caused by laser burns, and severe conditions, as in the case of cytomegalovirus retinitis and chronic uveitis. Although such conditions have been commonly treated with multiple intravenous or intravitreal injections, the development of intravitreal implants for controlled drug delivery has become the goal of many contemporary investigators (Jabs, 1992; Friedberg, 1995; Martin et al., 1994; Pearson et al., 1996; Sanborn et al., 1992; Smith et al., 1992). This was consistent with the need to eliminate, or at least minimize, the risk of nephrotoxicity associated with systemic administration. The fact that **cytomegalovirus (CMV) retinitis** is the leading cause of visual loss in patients with acquired immunodeficiency syndrome (AIDS)--affecting 20-40% of all patients (Martin et al., 1994)--has directed several investigators to the use of intravitreal, non-absorbable implants for the controlled delivery of ganciclovir. Such an implant was also investigated for the delivery of **cyclosporin A in treating chronic uveitis**. To acknowledge CMV retinitis while addressing the broader issue of effective administration of different bioactive agents to the inner eye using a minimally invasive procedure, the use of novel, injectable controlled-release formulations was sought. Thus, the use of novel absorbable gel- formers as drug carriers became the underlying theme of the present program. In addition to being easily administered to form a gel-like insert, it need not be surgically removed at the conclusion of treatment, the insert bioabsorbs through hydrolysis and elimination to safe by-products. Furthermore, having a compliant insert assures its mechanical biocompatibility with the ocular fluids and tissues. It is also expected that this formulation can be administered at the physician's office without need for hospitalization.

In addition to imminent need for a controlled release for treating CMV, the promising properties of the gel-former system were compelling, and exploring its use with other key drugs, having a range of physicochemical properties and different pharmaceutical attributes, was certainly justifiable. Thus, it was decided to extend the Phase I scope to include study on (1) cyclosporin A (CP) as a potent immunosupressant, and effect agent for treating chronic uveitis and a drug with distinctly low water solubility; (2) naproxen (NP) as a highly effective anti-inflammatory drug with yet-to-uncover potential valuable attributes as an ophthalmic drug which can be used in highly or slightly water-soluble forms (as sodium or free acid, respectively); and (3) pilocarpine (PC) as a model drug for use to non-invasively monitor grossly the release profile in the gel-former system and classical (not necessarily the most potent) antiglaucoma drug which is used in a highly water-soluble hydrochloride form.

Successful completion of Phase I study was intended, and was indeed shown, to provide the data for the comprehensive evaluation and development of at least one of four drug release systems and provide the ophthalmic community with an extraordinary, minimally invasive mode of effective administration of several valuable and pharmaceutically potent agents. Successful development of an injectable, absorbable pilocarpine (or more potent analogs), naproxen, ganciclovir and/or cyclosporin A can very well present

military and civilian patients with major milestones in the treatment of glaucoma, burn lesions, cytomegalovirus retinitis and/or chronic uveitis, respectively.

A.2. Technical Objectives of Phase I--The primary and three secondary **research questions** that were to be addressed in Phase I are given below:

1. Would selected members of a novel family of absorbable copolyester gel-formers be suitable as a safe carrier for the development of an injectable formulation for the controlled and effective delivery of valuable drugs to inner eye, particularly the retina?
2. Would it be feasible to use a novel gel-former carrier to deliver naproxen, cyclosporin A and/or ganciclovir to the retina and adjacent tissues of the rabbit?
3. Using pilocarpine gel formulations as subconjunctival and intravitreal injections and monitoring change in pupil diameter, can one compare the relative bioavailability of pilocarpine from these two administration sites?
4. Would it be possible to use pilocarpine subconjunctival injections in screening future gel-formers as candidates for intravitreal drug release?

Accordingly, the **technical objectives** of Phase I were: (1) review of available in-house release data on the performance of different members of the gel-former family as carriers for antibiotics, polypeptides, and proteins, and select the five most promising candidates for use in Phase I of this program; (2) preparation and characterization of the five candidate gel-formers; (3) preparation of formulations with candidate gel-formers, and determination of the drug release profile in a phosphate buffered medium (as outlined in Table I); (4) selection of slow- and fast-releasing gel-formers and their use to prepare, aseptically, injectable formulations of pilocarpine, napoxen, cyclosporin A, and ganciclovir; (5) completion of a statistically designed release and pharmacokinetic study using subconjunctival and intravitreal injections; (6) analysis of the *in vitro* and *in vivo* release and pharmacokinetic results and correlation with compositions of the systems to determine plans for Phase II study.

A.3. Related Work to Phase I Study and Its Clinical and Scientific Significance--This segment highlights related work on (1) ocular drug delivery systems; (2) role of biomaterials in contemporary ophthalmology; (3) absorbable polymers for controlled drug release; (4) absorbable gel and gel-like systems for pharmaceutical significance; and (5) key intraocular delivery systems.

A.3.1. Ocular Drug Delivery Systems--Historically, treatment of eye conditions has usually been effected through the use of applied ophthalmic drugs in either liquid or ointment form (Meadows et al, 1994). Such topical formulations are characterized by extensive drug loss, mainly due to efficient precorneal elimination. Additionally, they usually have poor patient compliance for reasons such as instillation difficulty and/or frequency of dosing. The Ocusert® system was developed to give controlled delivery of pilocarpine over a 7-day period but was not a commercial success because of the difficulty of instillation and the likelihood of ejection from the cul-de-sac during normal use (Meadows, 1994). In an attempt to address the clinical concerns associated with Ocusert®, Meadows and coworkers (1994) developed an ocular implant device that is surgically placed under the conjunctiva for prolonged delivery of drugs to the anterior region of the eye (Gwon & Meadows, 1992). The dosage system was an elliptically shaped unit containing a core reservoir of pilocarpine and alginic acid and surrounded by a hydrophobic ethylene/vinylacetate copolymer membrane plasticized by di-(2-ethylhexyl)phthalate. To evaluate the performance of the device, two studies were conducted. In the first study, Ocusert® was used as a model system containing pilocarpine and changes in pupil diameter was used as an indicator of pilocarpine release and activity. In the second case, a pharmacokinetic study was conducted to measure the tissue concentrations of pilocarpine when the device was either placed into the normal ocular cul-de-sac location or when it was subconjunctivally implanted. Timolol eye drops may cause systemic side-effects in

glaucoma patients due to absorption of the drug into systemic circulation. For this, Urtti and coworkers (1990, 1994) emphasized the need for developing a controlled delivery system for timolol and similar antiglaucoma drugs. These include the use of ocular inserts (Urtti et al, 1994) and viscous vehicles (Kyyronen and Urtti, 1990).

A.3.2. Role of New Biomaterials in Contemporary Ophthalmology--Most pertinent to contemporary ophthalmology is the modification of polymeric surfaces to increase the biocompatibility of implants including those used in controlled drug delivery as illustrated by the few examples given below. Corneal epithelial cell attachment and growth onto a synthetic corneal inlay is essential to the long-term incorporation of the prosthesis and the regeneration of a suitable refractive surface for the eye. Surface prosthesis was shown to increase all attachments and accelerate corneal epithelial healing (Garfinkle et al, 1994). Opacification of the normally transparent cornea is one of the leading causes of blindness. While vision is restored in many cases with corneal transplantation, the procedure is not always successful and often, tissue is not available (Leibowitx et al, 1994; Sheu et al, 1996). Surface-modified polyvinyl alcohol has been described as a useful alternative to natural tissue and its use in the production of artificial corneas was advocated as it allows for optimal epithelialization. Keratoprostheses are often extruded as a result of tissue melting, which in turn involves prolonged upregulation of the enzymes normally involved in tissue modeling, such as collagenase. While collagenase is essential for normal wound remodeling, over production or perpetuation of production leads to stromal degradation and implant loosening. Use of clinical regimes which reduce the initial collagenase response was suggested to reduce the risk of extrusion (Fitton et al, 1996). In their study on keratoprostheses, Fitton and coworkers (1996) concluded that anticollagenase regimes, such as those used topically for treating alkali burned cornea (Pfister, 1983) are a useful support in the initial stages of keratoprostheses implantation.

A.3.3. Absorbable Polymers for Controlled Drug Release--Absorbable (or often called biodegradable) polymers have been used clinically in sutures and allied surgical augmentation devices to eliminate the need for a second surgery to remove functionally equivalent non-absorbable devices (Schmitt, 1976; Shalaby, 1988). Although most of these devices were designed for repairing soft tissues, interest in using such transient devices, with or without biologically active components, in dental, orthopedic, and pharmaceutical applications has grown significantly over the past few years (Bezwada, et al., 1994; Bhatia et al., 1994; Damani, 1993; Dunn, et al., 1994, 1990; Schmitt, 1976; Shalaby et al., 1992b).

A.3.4. Absorbable Gels and Gel-like Systems of Pharmaceutical Significance--Prior to outlining the key features of the drug carrier subject of the proposed studies, the physical and chemical properties of known gels and gel-like materials are discussed below. In addition, Klech (1992) and others (Miyata et al, 1979) have reviewed many other gel formers which are available for preparing pharmaceutical gels.

A.3.4.1. Hydrogels--Hydrogels are materials which absorb water, undergo swelling without discernible dissolution, and maintain three-dimensional networks capable of reversible deformation (Park et al., 1993; Shalaby, 1991; Silberberg, 1989).

Covalently crosslinked networks of hydrophilic polymers, including water-soluble ones, are traditionally denoted as hydrogels (or aquagels). Hydrogels based on crosslinked polymeric chains of methoxy poly(ethylene glycol) monomethacrylate having variable lengths of the polyoxyethylene side chains, have been prepared and their interaction as hydrogels, with blood components has been studied (Nagaoka et al., 1983). A number of hydrogels are used in several biomedical applications such as soft contact lenses, wound management, and drug delivery. Methods used in the preparation of these hydrogels and their conversion to useful articles are subject to the constraints associated with the nature of their three-dimensional thermosetting structures and, hence, deprive the users from applying the facile processing techniques employed in the production of non-crosslinked thermoplastic materials. This, and the low

mechanical strength of the hydrated networks, led a number of investigators to explore the concept of combining hydrophilic and hydrophobic polymeric components in block (Okano, et al., 1981), or graft copolymeric structures (Onishi et al., 1984), and blends to form the so called hydrophobic-hydrophilic domain systems, which are suited for thermoplastic processing (Shah, 1991). It has been noted that the hydrophobic-hydrophilic domain system (HHDS) undergoes morphological changes which are associated with the hydration of the hydrophilic domains and formation of pseudo-crosslinks via the hydrophobic component of the system (Shah, 1991). Such morphology was considered to be responsible for the enhanced biocompatibility and superior mechanical strength of the two-phase HHDS as compared to those of covalently crosslinked, hydrophilic polymers.

A.3.4.2. Biodegradable Hydrogels--Synthesis and potential biomedical and pharmaceutical applications of absorbable or biodegradable hydrogels based on covalently crosslinked networks comprising polypeptide or polyester components as the enzymatically or hydrolytically labile components, respectively, have been described by a number of researchers (Jarrett et al., 1995; Pathak et al., 1993; Park et al., 1993; Park, 1988; Shalaby et al., 1992a). The most explored hydrogels are those made of water-soluble polymers, such as polyvinyl pyrrolidone, which have been crosslinked by naturally derived biodegradable components such as those based on albumin (Park et al., 1993; Shalaby et al., 1992a). Totally synthetic hydrogels, which have been studied for controlled drug release and as membranes for the treatment of post-surgical adhesion, are based on covalent networks formed by the addition polymerization of acrylic-terminated, water-soluble chains of polyether dl-polylactide block copolymers (Jarrett et al., 1995; Pathak et al., 1993).

A.3.4.3. Absorbable Hydrogel-Forming Carriers for Controlled Drug Delivery--Growing interest in developing absorbable pharmaceutical and surgical products which degrade in the biological environment to safe by-products and leave no residual mass at the application site (Shalaby, 1985a,b,c; 1988; 1991a, b, 1992a; 1994a,b,c; Shalaby & Shalaby, 1993), justified the search for novel, absorbable gels. In a recent disclosure (Shalaby, 1995), novel gel formers were described to be based on absorbable copolymers which, upon hydration, result in hydrogels that are stabilized by pseudo-crosslinks provided by hydrophobic polyester components covalently linked to hydrophilic ones made of pharmaceutically acceptable polymer, such as polyoxyethylene. The polyester component is made of safe monomers, such as p-dioxanone, e-caprolactone, glycolide, lactide, and mixtures thereof. Contrary to a related study (Dunn, et al., 1990), which describes *in-situ* formation of biodegradable, microporous, solid implants in a living body through coagulation of a solution of a polymer in an organic solvent such as N-methyl-2-pyrrolidine, the new hydrogel formers do not require the use of solvents. Such solvents did include low molecular organic ones that can migrate from the application site and cause damage to living tissue, such as cell dehydration and necrosis. Equally important is the fact that previously known systems are solid implants which can elicit mechanical incompatibility and, hence, patient discomfort as compared with the new compliant, swollen, mechanically compatible hydrogels (Shalaby, 1995). Meanwhile, potential applications of the *in-situ*-forming implants, and the more recent gel-formers, have been described to entail their use for tissue regeneration and release of growth factors (Dunn et al., 1994; Shalaby et al., 1995). Depending on the composition of the gel-formers used in the present study, these absorbable matrices can be used for the controlled release of antibiotics over a period of 1 to 6 weeks (Shalaby, 1995). In an ongoing study in mice, gel formulations containing Ricin A-Chain appears to effect its control release and generation of its seral antibodies.

A.3.5. Key Intraocular Delivery Systems--The main dosage forms of drug delivery to the eye are drops and ointment. For both forms, the duration of therapeutic effect is short lived, and repeated doses are required during the course of therapy because the drugs are rapidly washed out by reflex tear flow. Although several attempts have been made to use polymeric inserts, typically in the cul-de-sac, for prolonged release of drugs (Flach, 1993; Frangie & Leibowitz, 1993; Miyata et al, 1979) residence time of these inserts has been highly unpredictable. This directed many investigators to explore the use of

surgically implanted inserts. As will be seen below, most of these inserts still suffer from one or more drawbacks. A semi-solid biodegradable poly(orthoester) (POE) was investigated as a carrier for ocular delivery of 5-fluorouracil and mitomycin C in glaucoma filtering surgery (Merkli et al, 1993, 1994). To assess the biocompatibility of the polymeric carrier, Zignani and coworkers (1997) monitored the tissue reaction to subconjunctival injections of POE in rabbits. In the latter study, using New Zealand albino rabbits, a bleb was observed after a subconjunctival injection of 200 ml of POE. The bleb disappeared with three days. The injected polymer was reported to induce a moderate hyperemia of the conjunctiva and the episclera. The tissue fully recovered within two weeks. Histologically, no signs of encapsulation were observed. The two initial constituents and the degradation products induced a very light inflammation at a concentration of 50% (Wt/Wt).

Professor Gurny and his team explored the use of a solid bioadhesive insert, viscous polymer solution, and semi-solid polymeric insert as carrier of drugs for controlled ocular delivery (Gurtler et al, 1994; Merkli et al, 1994; Meseguer et al, 1994). In their study of the bioadhesive system, a special formulation was extruded into 2 mm single rods which were cut into 5 mm lengths. The solid formulation contained 40.2, 18.0, 1.8, 15.0 and 25.0 percent of a water soluble adhesive (Carbopol 93-4p, B.F. Goodrich), hydrophilic polymer (Klucel HSF-NF, Aquatin), a hydrophobic polymer (Ethocel N-50, Hercules), solid solution of solubility modifier (C.A.P., Fluka), and antibiotic (gentamicin sulfate, Ph.Eur), respectively. The inserts were placed in the conjunctiva of dogs and tear samples (4 ml) were collected over a period of three days. The tear samples were diluted with a isocryoscopic buffer and the analysis of gentamicin was performed on TDX (Abbott Labs) which makes use of fluorescent polarization technology in direct competitive binding immunoassay technique (Provost and Farinotti, 1984). Results indicated that an adequate concentration above the MIC (1.6 mg/ml) for most common infections is insured during three days.

Among the serious inner eye complications which are receiving substantial attention nowadays are the cytomegalovirus (CMV) retinitis, uveitis, glaucoma, and retinal lesions associated with intra-operative laser burns. CMV is the most common cause of viral retinitis in patients with AIDS, affecting up to 40 percent of patients (Smith et al, 1992). If left untreated, blindness inevitably results. Intravenous sodium ganciclovir is effective in treatment of CMV retinitis but requires frequent dosing. This often causes systemic side effects, including neutropenia, which necessitate treatment withdrawal in approximately one-third of patients. Other problems associated with systemic ganciclovir administration include sepsis related to permanent indwelling catheters and difficulties because of concurrent therapy with zidovudine. On the other hand, intravitreal ganciclovir injections provide a higher intra-ocular drug concentration than systemic drug therapy and reduce systemic exposure to the drug. Since the half life of ganciclovir is 13 hours, frequent injections are required to maintain therapeutic levels in the eye, intravitreal injections of 200-400 ml administered weekly have resulted in temporary remission of CMV retinitis. However, repeated injection can also lead to cataract formation, retinal detachment, and cystoid macular edema (Smith et al, 1992). For this, Smith and coworkers (1992) and others (Friedberg, 1995; Jabs, 1992; Martin et al, 1994; Sanborn et al, 1992) have explored or supported the use of an intravitreal non-absorbable implant for the controlled release of ganciclovir. The surgically placed implant was reported to be well tolerated by the eye.

Chronic uveitis, due to inflammation of the uvea, is a potentially blinding disease that requires frequent treatment. Cyclosporin A (CS) effectively inhibits the development of experimentally induced uveitis and is effective in suppressing auto-immune uveitis (deSmet & Nussenblat, 1993; Pearson et al, 1996). It was also shown in several human clinical trials that systemically administered CSA is effective in treating chronic uveitis (Pearson et al, 1996). Side effects from systemic administration including nephrotoxic effects and hypertension have led Pearson and coworkers (1996) to investigate the use of a surgically implanted intravitreal device for controlled release of cyclosporin A. The device is modeled after that described above for delivery of ganciclovir (Smith et al, 1992). Implanted intravitreally in a rabbit, a

device containing 5 mg of cyclosporin A (which has low solubility in the vitreous humor) was monitored over a six-month period. The device resulted in a vitreous concentration of 500 ng/ml throughout the study period. In the rabbit, it resulted in reversible lens opacification and decreased the b-wave amplitude. This toxic effect was not detected in rabbits. Nevertheless, Pearson and colleagues (1996) concluded that sustained release implants are a promising new treatment of uveitis.

For decades, glaucoma has been a common eye condition for which topical formulations such as those of pilocarpine have been used. Unfortunately, topical vehicles usually have poor patient compliance. These facts led a number of investigators to explore the use of controlled release devices such as the non-absorbable Ocusert® to be placed in the cul-de-sac. This was designed for a 7-day delivery. The large volume of the insert, the difficulty associated with its instillation, and the likelihood of ejection from the cul-de-sac led to the search for more effective alternatives (Meadows et al, 1994). Thus, Meadows and colleagues (1994) investigated the use of subconjunctival non-absorbable implants for the controlled release of pilocarpine. This implant was shown to provide a zero order release of the drug to anterior segment tissues in therapeutically effective concentrations over a period of 7 days.

Non-steroidal anti-inflammatory drugs (NSAID) have been widely used for the prevention and treatment of cystoid macular edema (CME) following cataract operations and in the management of post-operative ocular inflammation associated with cataract surgery (Flach, 1993). With increased use of lasers in eye surgery and increasing incidence of laser burns, contemporary investigators have called for the use of NSAID in sustained release mode for post-operative inflammations (Flach et al, 1993). Among the commonly used NSAID is flurbiprofen, although, a more effective NSAID such as naproxen is yet to be explored.

B. SUMMARY OF PHASE I ACCOMPLISHMENTS AND SIGNIFICANCE VERSUS INITIAL GOALS

In concert with phase I technical objectives, the pertinent experimental results and findings are summarized and contrasted with initial goals. Issues pertinent to problems encountered during phase I and corrective measures, as well as patents and publications are also included.

B.1. Summary of Phase I Accomplishments and Significance--Two injectable, absorbable, liquid gel-formers were identified and used for intravitreal and subconjunctival administration of active formulations. One gel-former was finally selected for conducting the comparative *in vitro* release study and *in vivo* evaluation in rabbit eye using the subconjunctival and/or intravitreal route of administration. This **verified** our postulate that the **gel-formers are suitable as vehicles** for injectable intraocular formulations.

Using **pilocarpine as a model** drug and probe, for gross evaluation of different effects associated with the administration of active and placebo formulations, led to the realization that: (1) up to 100 µl of gel-forming formulation can be injected without causing long-lasting, site adverse reaction; (2) up to 1 mg, and preferably 0.5 mg, of pilocarpine can be administered and controllably released without inducing long-lasting site adverse reaction; and (3) no significant difference in drug release was detected between intravitreally and subconjunctivally administered formulations. This indicates the **availability of two administration sites** for introducing gel-forming drug formulations at higher doses than clinically required.

Using a continuous-flow cell with a flow rate of 47 µl/hr, the release profiles of four different drugs into a phosphate buffer at 37°C, pH 7.2, were determined. Pertinent ***in* vitro release data** indicate that (1) at a drug **loading of 5%**, the initial release profile (during the first week) decreases in the following order: ganciclovir > naproxen > pilocarpine >> cyclosporin A--50% or more of naproxen and ganciclovir, and

less than 40% of pilocarpine were released in the first week; (2) at four weeks, **less than 20%** of cyclosporin A is release from the 5% formulation; (3) at 8-10% drug loading, the release profile during the first week decreases in the following order: naproxen >ganciclovir > pilocarpine >> cyclosporin A--50% or more of naproxen and ganciclovir and less than 40% of pilocarpine are released in the first week; and (4) at four weeks, **less than 10%** of cyclosporin A is released from 8% formulation. This **reflects** the great **dependence** of the release profile **on drug polarity, loading,** and solubility and the exceptionally **slow** release profile of cyclosporin A.

The normalized ***in vivo* release data,** based on drug analysis in retrieved gel formulations from 3-5 animals for each period, indicate that (1) at 5 days, about 98, 97, 89, and 45% of pilocarpine, naproxen, ganciclovir, and cyclosporin A were released, respectively; (2) at 14 days, the apparent (based on normalized percent of drug remaining in coherent retrievable, residual gel mass) percent of **cyclosporin A** released is about **38%**; and (3) **ganciclovir** continues to release steadily for a **period of 21 days**. Compared with *in vitro* data, the *in vivo* results show that (1) the drug release rate is faster regardless of drug type; (2) a similar dependence of the release profile on drug solubility in water prevails; (3) in the case of pilocarpine and naproxen, the release rates do not decline considerably beyond one week, but continue steadily to almost 100%; (4) for ganciclovir, *in vivo* release profiles approach that of *in vitro* ones at 5% loading; (5) **the *in vitro* release methodology** can be used **for screening** different formulations to uncover certain trends, develop relative releasing rate data, and predict, in some instances, general *in vivo* release profiles and, in a few cases, the specific profiles; and (6) *in vitro* screening can be used to design a more effective *in vivo* release study, but will not be a viable substitute therefore.

Gross observation of rabbit eyes treated with the placebo and active formulations and **histological evaluation** of the selected sites show that (1) 50 or 100 µl of placebo and active formulations containing 10% pilocarpine, ganciclovir, or naproxen can be easily injected intravitreally or subconjunctivally without causing any discernible inflammation at the injection site beyond the initial traumatic reaction; and (2) reaction to cyclosporin A at 8% concentration was more pronounced than those of the other three drugs at 10% loading. **Thus for three drugs**, there appear to be a **broad dosing window**. However, **for cyclosporin A, only low doses** should be considered in future studies.

Collectively, it can be concluded that the (1) gel-former technology is useful for the development of intraocular drug delivery systems based on active agents having a broad range of properties; (2) gel-formers can be used not only for intravitreal, but also subconjunctival, therapeutically effective formulations; (3) continuous flow-cell system, designed and employed in monitoring the *in vitro* release profile, can be used for accurate comparative study of the *in vitro* controlled release of different formulations to allow for a timely modulation of the gel-former compositions in order to attain an optimum profile for succeeding *in vivo* studies; (4) Phase I data can be used to pursue further the development of, at least, one drug beyond the safety study stage; (5) established dependence of the release profile on drug solubility and polarity may be utilized for exploring the application of this technology beyond the drugs used in Phase I; (6) gel-former technology may be extended to indications of military and civilian significance, such as corneal injuries and ulcers; (7) adhesive and flow characteristics of the formulations examined in Phase I may allow the use of the gel-formers in these formulations to be administered at the cul-de-sac; and (8) highly potent drugs may be well suited for delivery using gel-forming formulations with or without a microparticulate cation-exchanger.

B.2. Achievements versus Goals--Contrasting the accomplishments noted in Section B.1. with Phase I technical goals as described in Section A.2. allows one to make the following conclusions: **Phase I accomplishments do satisfy the initial goals of the study and provide additional information** that is most pertinent to the successful development of this technology in Phase II.

B.3. Identified Problems and Corrective Measures--Of the identified problems addressed in the monthly reports, those given below are most important.

1. To maximize the outcome of the study, the animal protocol was revised to up-scale the pilot study. This required amending the protocol and purchasing more animals.

Corrective Measure--The animal protocol was amended and approved on a timely basis. The additional animal cost was absorbed within the Poly-Med budget.

2. The continuous flow system for monitoring the *in vitro* release was shown to be inconsistent upon using the originally planned slow flow rate.

Corrective Measure--The system was immediately modified, substantially, to provide a faster flow rate and minimum or no mechanical interruption.

3. Having a local veterinarian ophthalmologist was deemed critical for a timely execution of Phase I study.

Corrective Measure--Dr. Martin of the University of Georgia was invited to join the project as a major collaborator. His contribution to the program was timely and most valuable.

4. Using pilocarpine at the planned low dose levels as a model drug in our efforts to select the proper dose level for other drugs and administration site was impractical for attaining discernible biological responses.

Corrective Measure--The dose was increased substantially to achieve measurable responses.

5. Net concentrations of release drug in eye tissues were too low for reliable detection and use in determining drug levels in different eye components and the overall release profile.

Corrective Measure--A protocol was developed to retrieve coherent , tangible gel masses from the vitreous humor for analysis of residual drug and indirect determination of the fraction of released drug.

6. The available HPLC protocols for analysis of the different drugs needed time-consuming modification. This was particularly troublesome with cyclosporin A, where the available protocol needed substantial refinement to obtain reliable results.

Corrective Measure--A carefully planned schedule was established for continuous use of the HPLC units for analytical method development and routine data collection.

B.4. Patents and Publications--

- U.S. Patent No. 5,612,058, which covers the gel-forming absorbable technology, was issued.
- A U.S. patent application covering the novel flow-cell system is being prepared.
- A divisional U.S. patent application on the use of the gel-formers was filed.
- The PI presented a paper at the IUPAC meeting in Stockholm in which he presented some of the release data.
- A paper and meeting abstract are being prepared for submission within the next three months.

C. MATERIALS AND METHODS

C.1. Materials--This section deals with (1) a description of the four drugs used; (2) the types of gel-forming copolymers used as matrices; and (3) the different monomers, polymers, chemical reagents, and solvents used in the polymer synthesis and analytical procedures.

C.1.1. Drugs investigated

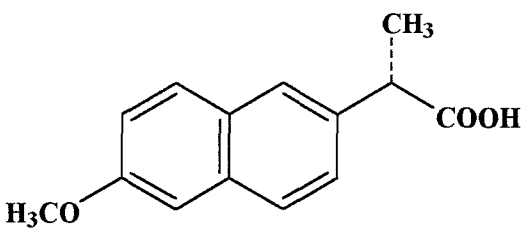
1. Pilocarpine hydrochloride (Sigma, CAS Registry # [54-71-7]),

2a. [+]-Naproxen: (S)-6-methoxy-α-methyl-2-napthaleneacetic acid, (Sigma, CAS Registry # [22204-53-1])

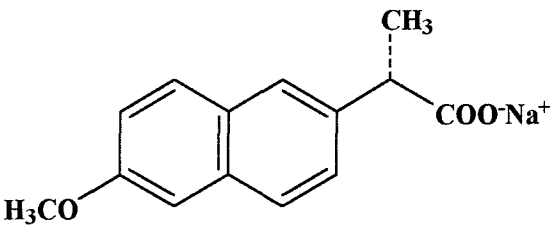
2b. [-]-Naproxen sodium: (S)-6-methoxy-α-methyl-2-napthaleneacetic acid sodium salt, (Sigma, CAS Registry # [26159-34-2])

3. Ganciclovir sodium: 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine, monosodium salt, (Roche)

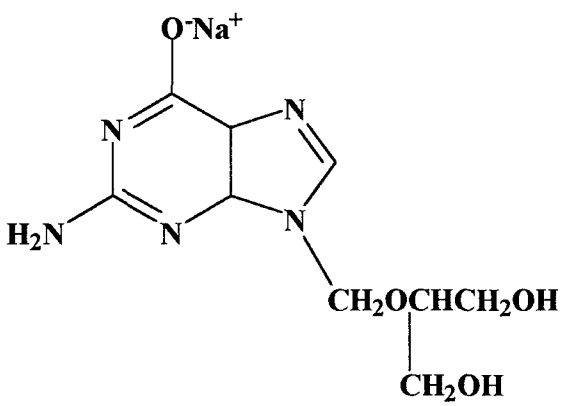
4. Cyclosporin A (Sigma, CAS Registry # [59865-13-3])



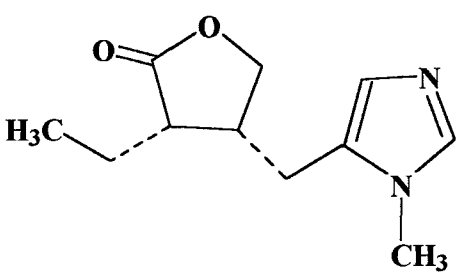
Naproxen



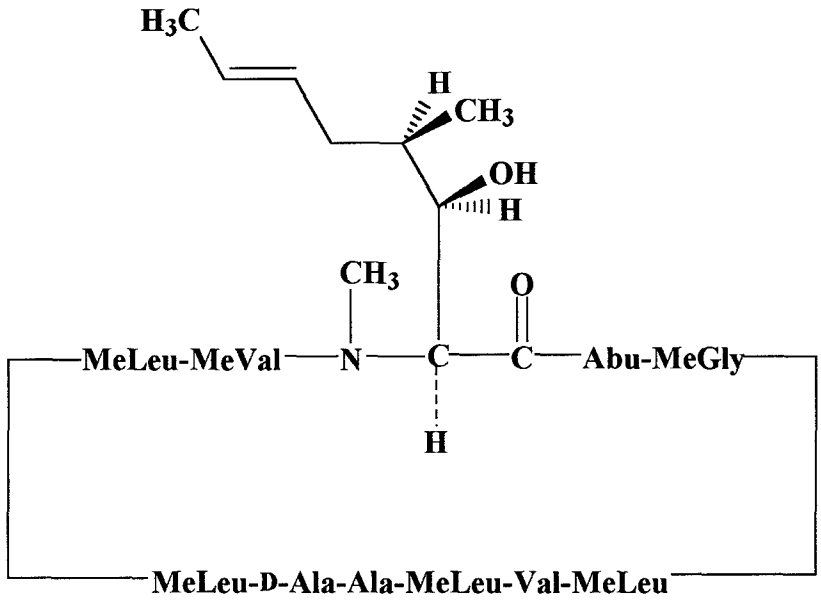
Naproxen sodium



Ganciclovir sodium



Pilocarpine



Cyclosporin A

C.1.2. Types of Gel-Formers--Two selected gel-forming combinations, namely GF-55 and GF-64 were based on two primary gel-forming copolyesters, GF-01 and GF-02 at GF-01/GF-02 ratios of 50/50 and 60/40, respectively. GF-01 is a relatively hydrophobic gel-former (as compared to GF-02) comprising more than 60% and less than 40% of poly(dl-lactide-co-glycolide) and polyethylene glycol 400 segments, respectively. On the other hand, GF-02 is a relatively more hydrophilic gel-former (as compared to (GF-01) comprising more than 60% and less than 40% of polyethylene glycol 400 and poly(dl-lactide-co-glycolide), respectively.

C.1.3. Monomers, Pre-Polymers, Chemical Reagents, and Solvents--Monomers and gel-formers intermediates used in this study were *dl*-lactide (Purac, Inc.), glycolide (NORAMCO, Inc.), polyethyleneglycol 400 (Aldrich), glycolic acid (Aldrich), and stannous octoate (Sigma-Aldrich). Trifluoroacetic acid, TFA, (Aldrich), acetonitrile, ACN, (Sigma), tetrahydrofuran, THF, (Sigma-Aldrich), chloroform, $CHCl_3$, (Sigma-Aldrich), deuterated chloroform, $CDCl_3$, (Aldrich), tetramethylsilane, TMS, (Aldrich),Tears Naturale® eyedrops (Alcon, Inc.), sodium hydroxide (Aldrich), and Davidson's fixative (contains 1320 ml of 95% ethanol, 880 ml of 10% neutral buffered formalin, 440 ml of glacial acetic acid, and 1320 ml of deionized water.

C.2. Methods--This section deals with methods and/or instruments used in (1) preparing and evaluation of gel-formers; (2) preparing the drug formulations; (c) conducting the *in vitro* release studies; (4) administering of the drug formulation into rabbit eye; (5) tissue and gel-formulation retrieval; (6) analysis of residual drug in retrieved gel-formulations; (7) histological evaluation of retrieved tissues; and (8) instrumental analysis.

C.2.1. Preparation and Evaluation of Gel-Forming Polymers--Gel-formers GF-01 and GF-02 were prepared by grafting a mixture of dl-lactide and glycolide onto polyethylene glycol in presence of stannous octoate as a catalyst. The polymerization was conducted under typical ring-opening conditions as described by Shalaby (1997). The copolymers were analyzed for molecular weight and molecular weight distribution, chemical identity, and compositional purity using gel-permeation chromatography (GPC) of

polymer solutions in tetrahydrofuran (THF), Fourier-transform infrared (FTIR), and nuclear magnetic spectrophotometry (NMR), respectively. The flow properties of the different gel-formers were evaluated in terms of ease of injectability through a 27 gauge needle.

C.2.2. Formulation of Drugs--Homogenous mixtures of the hydrophilic and hydrophobic components of the carrier gel in different proportions were prepared. Since the particle size of the loaded therapeutic agent can have a drastic effect on the diffusion, a sieving method was developed and utilized to ensure that no particle was over 125 microns. This was accomplished by grinding the drug in a mortar and sieving it through a 120 mesh screen (screen aperture of 0.125 μm). After the correct amount for the required drug loading was calculated and weighed, the ground drug was loaded into the viscous carrier matrix using a slow speed electric mixer that uses a stainless steel paddle to mix the formulations uniformly. Thorough mixing is an extremely important step in ensuring uniform release from the formulation.

For use *in vivo*, additional precautions are taken to ensure the sterility of the formulation. The gels are routinely handled in a sterile manner after preparation. Drug loading is done in a laminar flow hood after wiping down with 70% isopropyl alcohol (IPA). Immediately before use, the hood and all equipment that is to be used in the hood is subjected to at least 30 minutes of UV sterilization via a lamp that is designed for this purpose.

The actual drug form chosen for each of the therapeutic candidate was carefully considered. **Pilocarpine hydrochloride** was loaded as a ground, sieved, white, micro-crystalline powder. **Cyclosporin A** was loaded as a ground, sieved, white powder. **Ganciclovir sodium** was supplied by prescription as Cytovene IV®, which comes in the form of a lyophilized, white powder to be reconstituted in situ with 10 cc of USP-grade water immediately prior to injection. However, for incorporation into the carrier gel formulation, the ganciclovir powder was taken from the prescription vial after removal of the septa cap, ground in a mortar, sieved, then added to the gel formulation prior to mechanical mixing. **Naproxen** was loaded into the gels as a **1:1 mixture** by weight of the **acid form and its sodium salt**. This ground, intimate 1:1 mixture was sieved before mixing with the gel formulation.

C.2.3. *In vitro* Release Study Methods

C.2.3.1. Overview--To examine the release of the drug formulations *in vitro,* a **special flow cell** was developed (to replace an older design with a slow flow rate of 30 μl/hr, which was noted for its inconsistent performance). Fifty milligrams of the drug formulation was loaded into a special flow cell. Phosphate buffer at pH 7.2 was introduced into the flow cell and flowed tangentially over the gel former in its representative area. The entire flow cell is kept in an incubator at 37°C and the flow rate is 47 μl/hour. The buffer is collected in a refrigerated environment and the collection vessels are changed at least every 48 hours. The weight of the collected liquid is determined. The samples are then frozen until analysis. (See Section C.2.3.4.)

C.2.3.2. Preparation of Buffer Solution for Continuous Flow *In Vitro* Release Studies--This phosphate buffer solution was made in 500 ml lots, and once prepared, were stored in a 4°C refrigerator. Each 500 ml solution was prepared by adding 11.4 g of dibasic potassium phosphate (K_2HPO_4), 3.1805 g mono-basic potassium phosphate (KH_2PO_4), and 2.5 ml of a 10% wt/vol solution of sodium azide (NaN_3) to a flask, and adding ultrapure water to achieve a total volume of 500 ml. This solution was well mixed, and tested to verify a pH of 7.2 ± 0.05.

C.2.3.3. HPLC Analysis Overview

C.2.3.3.1. HPLC Method Development--The methods for pilocarpine and for naproxen was developed by reviewing the USP method and then adapting the method for the available HPLC system. The method had

to be optimized to use the minimum amount of time and solvent due to the large volume of calibration, samples and analysis that are necessary to generate a release curve. However, the retention time of the analyte had to be sufficient to allow the gel breakdown products to flow through the column so that they would not interfere with the analyte peak and subsequent integration.

C.2.3.3.2. HPLC Calibration for Each Analyte--In order to determine the amount of analyte in each drug, the optimized method is used to run a series of dilutions to prepare a calibration curve. If the correlation coefficient for a linear fit was not at least 0.99 then the curve was regenerated with fresh dilutions of analyte.

C.2.3.3.3. Drug-Specific HPLC Protocols--An HPLC method was developed for each of the four therapeutic agents, and these methods, respectively, were applied to dilution series of known concentration analyte solutions in order to generate the necessary calibration curve. Then the release study samples were analyzed by the same method. The methods developed for pilocarpine, naproxen, and ganciclovir with the method for analysis of cyclosporin A being the most problematic of the group. The pertinent specifications of these methods are given below:

Pilocarpine HCl HPLC Method
Solvent A: 0.1% TFA in water
Solvent B: 0.1% TFA in acetonitrile
Column: Vydac 201TP104 (C18 chemistry)
Flow Rate: 1.5 ml/min; Detection at 220 nm
100µl sample loop and 10µl injections
Gradient: 5 to 40% B over 7 minutes
Column equilibrated for 7 minutes before analysis

Naproxen HPLC Method
Solvent A: 0.1% TFA in water
Solvent B: 0.1% TFA in acetonitrile
Column: Vydac 201TP104 (C18 chemistry)
Flow Rate: 1.5 ml/min; Detection at 254 nm
100µl sample loop and 10µl injections
Isocratic: 50% A/50% B over 6 minutes
Column not equilibrated before analysis

Ganciclovir HPLC Method
Solvent A: 0.1% TFA in water
Solvent B: 0.1% TFA in acetonitrile
Column: Vydac 201TP104 (C18 chemistry)
Flow Rate: 1.5 ml/min; Detection at 254 nm
100µl sample loop and 10µl injections
Isocratic: 99% A/1% B over 6 minutes
Column not equilibrated before analysis

Cyclosporin A HPLC Method
Solvent A: water
Solvent B: acetonitrile
Column: Vydac 218TP104 (C18 chemistry)
Flow Rate: 1.5 ml/min; Detection at 254 nm
100µl sample loop and 10µl injections
Isocratic 70% B for 2 minutes, then
gradient of 70 to 100% B over 3 minutes,
then isocratic with 100% B for 3 minutes
Column equilibrated for 9 minutes before analysis

C.2.3.4. Release Curve Development--The samples that are collected are thawed out and aliquots are run on the HPLC from the longest to the shortest time point. This is done to ensure the lowest concentration peaks are run first and will not interfere with subsequent peaks. The peaks are integrated and the areas are used to calculate the amount in the injection volume. A check is performed to ensure that the area from the chromatogram is within the linear range of the standard curve. This is done to ensure that the calculations for release are not over or under calculated due to non-linearity of the release curves at high and low concentrations. If the area amount was too great, then the sample was diluted and reanalyzed by HPLC and the amount was calculated to account for the dilution. The total amount of material that was released during the time period over which the sample was collected is calculated. The total amount of analyte released is plotted against the time point to prepare the release profile.

C.2.3.5. Flow Cell Development--The development of a new system for continuous flow drug release studies was crucial in determining the release profiles and characteristics of the various loaded gels. This system uses disposable, off-the-shelf items, is consistent and dependable. The flow rate of the phosphate

buffer solution (150 mmol/L, pH 7.2) over the exposed carrier gel matrix was approximately 47 µl per hour. The flow was collected into test tubes in a 4°C environment at, and the collection tubes changed at least every 48 hours. This continuous flow release cell system is inexpensive, easy to assemble, and virtually maintenance-free. As such, its commercial value is significant to researchers desiring a reliable way of studying dynamic as opposed to static *in vitro* release of bioactive materials. Poly-Med, Inc. Is preparing a patent application for this pharmaceutical research device and associated systems.

C.2.4. *In Vivo* Release Study

C.2.4.1. Overview--In the ocular application of the gel-forming systems, New Zealand White Rabbits were injected with various drugs and gels to examine the toxicity of the materials. Two placebo gels of 60/40 and 50/50, GF-02/GF-01, compositions were injected intravitreally to determine their compatibility. Both gels were deemed compatible, but GF-64 was more difficult to inject due to its higher viscosity, as compared to GF-55, in the 27 gauge needle used. Pilocarpine was loaded into the GF-55, and subconjuctival and intravitreal injections were performed on eight rabbits. It was determined from this that the intravitreal site would be more appropriate for monitoring the drug efficacy than the subconjunctival one.

In the next step, four rabbits were injected with 5% formulation of ganciclovir, naproxen (as 50/50 free acid/sodium salt), cyclosporin A, and pilocarpine. The rabbits were euthanized at four days post-injection and the eyes removed. The gel was retrieved for residual drug analysis and the eye dissected and all tissues were stored and frozen for possible tissue analysis.

In order to **test the extraction efficiency**, a non-implanted 0.7% pilocarpine formulation (50.1 mg) was dissolved in 500 µl acetonitrile (ACN). An equal amount of water was added to the ACN gel solution. An aliquot was run on the HPLC and the amount of pilocarpine present was calculated. Theoretically, the solution concentration was 0.350 mg/ml using 50.1 mg of 0.7% pilocarpine formulation in 1 ml of 50/50 ACN/water. In a similar way, subconjuctival gels, based on initial pilocarpine concentrations of 0.236 and 0.7% that were removed from the rabbit eyes were analyzed for residual pilocarpine. This was performed by homogenizing the gel/tissue in acetonitrile to solubilize the gel and then adding an equal amount of water to the mixture while still in the homogenizer. The mixture was centrifuged and the supernatant was examined for pilocarpine content. No pilocarpine was evident by this method in either gel. The 0.236% gel had been implanted for 34 days and the 0.7% gel had been implanted for 27 days. This was used to propose that the drug was completely released from both gels at these periods, or the HPLC method is not sensitive enough to detect trace drug concentrations.

C.2.4.2. Administering Drug Formulations into the Rabbit Eye--The rabbits were anesthetized and the formulation was injected into the desired site using a 27 gauge needle. For a conjunctival injection, the conjunctiva was lifted with forceps and the formulation injected directly underneath this layer. For a vitreal cavity injection, the needle was inserted between the jaws of the forceps and into the globe in such a fashion that the needle was angled away from the lens. This was done to ensure that the positioning of the gel was in the center of the globe and away from the retina and the lens. The needle was removed and the forceps was used to close and seal the needle hole and prevent reflux.

C.2.4.3. Tissue/Gel Retrieval--Immediately after the rabbits were euthanized, the eyes were quickly harvested and labeled OD (ocular, dexter) or OS (ocular, sinister). The eyes were placed in specimen containers and frozen at -80°C. The eye samples were later thawed and the globe was halved with a scalpel. The partial frozen vitreous fluid was collected and allowed to undergo complete thawing. Any tangible, coherent gel mass was removed via careful manipulation with tweezers and placed in a weighed vial. To remove all eye fluids from the gel sample, the vial was placed in vacuum oven at room temperature (to

protect the drug). After at least two hours of evacuation to reach a constant weight, the vial was weighed and the amount of gel formulation collected was calculated.

C.2.4.4. Extraction and Analysis of Retrieved Gel Formulations--Each of the four drug-containing gel formulations required a distinct extraction procedure. The efficiency of each extraction procedure was determined by extracting a control gel of known drug concentration. This value, or percent efficiency, was then used to calculate the actual amounts of drugs remaining in the *in vivo* gel samples.

The gels obtained after *in vivo* release were removed from the dissected ocular specimens and manually transferred via scalpel and forceps to Eppendorf® micro-centrifuge tubes. The particular extraction protocol necessary was then performed on the gel sample within the microcentrifuge. After the extraction procedure was performed, a sample aliquot was removed to an HPLC vial for chromatographic analysis.

C.2.4.4.1. Pilocarpine Extraction Protocol--HPLC-grade acetonitrile (250 µl) was added to the microcentrifuge tube containing the gel specimen, then subjected to vortex mixing for 30 seconds. Next, 750 µl of a 0.1 N Na_2CO_3 solution was added to the Eppendorf® and the contents vortex mixed for an additional 30 seconds. The microcentrifuge tube was spun down at 13,000 rpm for 10 minutes, and an aliquot removed from the supernatant for subsequent analysis. This procedure was identically performed on control gels, giving a 77.82% extraction efficiency because of the high solubility of CS in the aqueous ACN.

C.2.4.4.2. Ganciclovir Extraction Protocol--HPLC-grade acetonitrile (200 µl) was added to the gel specimen in a microcentrifuge tube and vortexed for 30 seconds to dissolve the gel. Then, 200 µl of a 1M NaOH solution was added and tube vortexed for an additional 30 seconds before being spun down at 13,000 rpm for 10 minutes. An aliquot was removed from the supernatant for analysis. Based on extraction of control gels, this method has a 19.06% extraction efficiency.

C.2.4.4.3. Cyclosporin A Extraction Protocol--HPLC-grade acetonitrile (500 µl) was added to the microcentrifuge tube and allowed to stand for 10 minutes, before the addition of 500 µl of distilled water. The Eppendorf tube was vortex-mixed for 30 seconds, then spun down at 13,000 rpm for 10 minutes. A portion of the supernatant liquid was removed for analysis. As no control gel was available to test, the method was assigned a 100% extraction efficiency.

C.2.4.4.4. Naproxen Extraction Protocol--HPLC-grade acetonitrile (400 µl) was added to the gel sample in the microcentrifuge tube and vortexed for 15 seconds, then 400 µl of 0.1N Na_2CO_3 was added, and the tube vortexed for 3 minutes, then spun down on the bio-centrifuge at 13,000 rpm for 10 minutes. A sample for analysis was removed from the supernatant liquid. Analysis of control gels with known concentrations of naproxen give this method a 59.91% extraction efficiency.

C.2.4.5. Histological Evaluation of Retrieved Tissue--Eyes that were to be evaluated histologically were placed in Davidson's fixative solution immediately after harvesting to properly preserve the specimen until histological analysis. The preserved specimens were later transported to the Pathology department of the University of Georgia in Athens, Georgia.

The specimens were received preserved in Davidson's fixative solution and taken to the necropsy room for processing. The air vents were turned on to circulate air flow and prevent concentration and exposure to formaldehyde. The work area was prepared by collecting the cutting table, blades, blade holder, yellow blocks (tissue holder), bottle of formulin for use on the blocks, and metal clamp that secures blocks. Each block was numbered with an appropriate pathology number.

The dissection was initiated by identifying the optic nerve protruding from back of the eyeball, then trimming excess tissue away from the eyeball without cutting the optic nerve. The long posterior ciliary artery (which traverses from the optic nerve around the equatorial line to the lens, on both sides of the eyeball) was located. The incision was made into the eyeball perpendicular to the imaginary plane bounded on each side by the long posterior ciliary artery and parallel to the closest line distance between the optic nerve and the lens. The cut was made through the eyeball including the lens, rendering two halves of the eye. The eye tissue and lens were placed in one of the yellow, numbered blocks, and the metal clamp applied to secure the tissue in the block. The blocks with specimens were immersed in formalin.

After dissection the blocks with specimens were taken to the histopathology lab for processing and fixing tissue in paraffin. Then, a microtome was applied to the ocular specimens generating thin slices. These slices were stained and subjected to observation via optical microscope.

C.3. Instrumental Analysis

C.3.1. High Performance Liquid Chromatography (HPLC)--The HPLC system used for these studies is a new Rainin Dynamax model. It consists of two Model SD-200 pumps, an automatic sample injector Model AI-3, and a UV-C absorbance detector. It is controlled by a Dell Dimension XPSP100c Pentium computer. The system is capable of injecting 96 samples automatically. The column used for pilocarpine, naproxen, and ganciclovir is a Vydac C18:201TP104 that is always run in the reverse phase mode. The column used for Cyclosporin A analysis is a Vydac C18: 218TP104 that has only been used for cyclosporin A and has not been used with any solvent containing TFA. Any methods that are not isocratic (containing a gradient) contain at least a seven minute equilibrium step to enable the column to return to its original state.

C.3.2. Fourier Transform Infrared (FTIR) Spectroscopy--A Perkin Elmer Model 1000 PC Paragon FTIR spectrometer was used to evaluate gel formers during their preparation. Samples were dissolved in chloroform, then the solution cast on a salt plate and the solvent allowed to evaporate before analysis.

C.3.3. Nuclear Magnetic Resonance (NMR) Spectroscopy--Gel former samples were analyzed by proton and ^{13}C NMR after preparation on a 300 MHz Bruker FT-NMR spectrometer. Samples were dissolved in deuterated chloroform as the lock solvent with 1% tetramethylsilane (TMS) as an internal standard.

C.3.4. Gel Permeation Chromatography (GPC)--Molecular weight determination and purity of the carrier formulations were determined using a Waters Associates gel permeation chromatograph using THF as the mobile phase.

C.3.5. *In Vitro* Release Study Pump--Pumping of the buffer in the release studies was accomplished by an Ismatec IPC 8 channel peristaltic pump.

C.3.6. *In Vitro* Release Study Incubator Shaker--For the duration of each individual *in vitro* study, the release cells were maintained at 37°C within a Controlled Environment Incubator Shaker Series 25 (New Brunswick Scientific, Inc.).

C.3.7. Vortex Mixer--Gel extraction protocols were facilitated by the use of a Vortex Genie 2, model G-560 (Scientific Industries, Inc.) to speed up solvation steps in the extraction procedures.

C.3.8. Vacuum Oven--A 1400E Model Vacuum Oven (VWR Scientific Products, Inc) was used to dry gels before extraction and to ensure moisture-free monomers and reagents.

C.3.9. Biological Centrifuge--A Biofuge 13 (Heraeus Instruments, Gmbh) centrifuge was used as part of the gel extraction protocols for this study.

D. EXPERIMENTAL RESULTS AND DISCUSSION

Accomplishments and results of Phase I tasks are discussed below.

D.1. Polymer Synthesis and Identification of Candidate Gel-Formers

D.1.1Review of Earlier Release Profile Data--Release profile data of different active compositions from a number of gel-forming systems were reviewed to identify two primary gel-formers for preparing injectable candidate matrices for the present study. The reviewed systems dealt with the release of proteins, oligopeptides, and antibiotics (e.g., vancomycin). Two primary gels, which are mutually miscible at all proportions, were identified. Specifically, slow-absorbing, highly viscous, moderately hydrophobic (GF-01) and relatively fast absorbing, low viscosity, more hydrophilic (GF-01) gel-formers were identified for mixing to produce formulation matrices suitable for injection using a 27 gauge needle.

D.1.2. Synthesis and Characterization of Primary Gel-Formers--the two identified gel-formers, GF-01 and GF-02 were prepared and characterized. GF-01 was designed to be a relatively more hydrophobic, less absorbable, higher molecular weight polymer with a slower releasing matrix as compared to GF-02.

D.1.3. Identification of Injectable Gel-Formulations--Several combinations of the primary gel-formers (GF-01 and GF-02) were prepared and evaluated, mainly for ease of gel-formation and injectability from a 27 gauge needle. Of all the examined combinations, GF-55 and GF-64 having GF-01/GF-02 ratios of 50/50 and 60/40, respectively, were selected as two final candidate injectable gel-formulations. As expected, GF-55 was easier to inject than GF-64, but the latter formed a firmer gel.

D.1. 4. Pilot *In Vitro* Release Study and Selection of One Gel-Forming System

D.1. 4.1. Pilot *In Vitro* Release Study--High performance liquid chromatography (HPLC) protocols for analyzing pilocarpine and naproxen in phosphate buffered solutions were developed and the respective standard curves were prepared. These were used later in monitoring the *in vitro* release profile of these drugs from candidate gel-forming formulations.

A major component of this segment dealt with assembling a continuous flow system for the continued monitoring of the different drugs from gel-forming matrices. This system was designed to (1) allow a stream of buffered solution at pH 7.2 to contact tangentially a micropool of drug formulation along its surface; and (2) allow the modulation of the flow rate to accelerate the drug release if so desired. The basic components of the system are (1) a specimen compartment kept at 37°C; (2) an 8-channel peristaltic pump; and (3) refrigerated receptacles (to assure drug stability in the collected effluent solution).

D.1. 4.2. Selection of One Gel-Forming System--To achieve this task, the two final candidates, GF-55 and GF-64, were evaluated for the release profile of a model drug (e.g., pilocarpine) and injectability of the active formulations from a 27 gauge needle. As noted earlier for the placebo formulation, GF-55 was easier to inject than GF-64. Using the HPLC protocol for analyzing pilocarpine, the release profile of two gel-formers containing 10% of this drug were determined. This was pursued using the continuous-flow system at a relatively high rate (to minimize time on task). The resulting data did not reflect a significant difference between the two formulations in terms of their release profiles. Subsequently, it was decided to select the more fluid system, GF-55, for use in the balance of Phase I study.

D. 2 Main *In Vitro* Release Studies

D.2.1. Development of the HPLC Analytical Protocol of the Four Drugs--All HPLC studies were conducted using acetonitrile/water as the mobile phase and reverse phase columns. The studies entailed (1) revision of the pilocarpine and naproxen protocols noted in Section C.2.2.1. using a C_{18} reverse phase column and establishing the respective standard curves; (2) development of the protocol for ganciclovir using a C_{18} column; and (3) using a C_8 column to develop the cyclosporin A HPLC protocol. The latter protocol was most difficult to develop due to the very low solubility of cyclosporin A in aqueous systems, which required the use of higher acetonitrile concentrations in the mobile phase.

D.2.2. Establishing the *In Vitro* Continuous Release Protocol--An 8-cell system with 50 mg sample compartment attached to a peristaltic pump was used. In all cases, the gel-former formulation was allowed to contact tangentially and continuously a phosphate buffer solution at 37°C in pH 7.2. The effluents were collected in receptacles and kept at 4°C. After collection, the samples were frozen until performing the HPLC analysis. Although few of the initial experiments were conducted using a buffer flow rate of 30 µl/hr, all the final *in vitro* release results were based on a flow rate of 47 µl/hr. This artificially accelerated release rate was used to economize on the experimental time, while realizing that data should be used in a relative manner upon comparison with the corresponding *in vivo* experiments. In all cases, at **least two drug concentrations** were used. In turn, in all cases, each concentration was run in **duplicate or triplicate**.

For pilocarpine, ganciclovir, and naproxen, 5 to 10% drug concentrations in the gel-former were used. For cyclosporin A, 5, 8, and 10% concentrations in the gel-former were used. Only the selected gel-former (GF-55) was used in the final *in vitro* release studies, as well as the main *in vivo* studies. The gel-former selection and drug loading was based, in part, on the results of the pilot *in vivo* studies (to determine, grossly, the maximum safe dose and formulation volume) and the detection limits of the HPLC analytical procedures. The *in vitro* release profiles were based on at least two-week and, in some cases, more than four-week studies.

D.2.3. Results of the *In Vitro* Release Studies--The release data of the four drugs are summarized in Table I and illustrated in Figures 1 to 4. The average release data are based on duplicate and triplicate runs of 50 mg formulations. Release data of 5% and 10% **pilocarpine (PC as the hydrochloride) formulations** are shown in Figures 1a and 1b and Table I. The results indicate that about 30% and 45% of the drug in the 5 and 10% formulations are released during the first week, respectively, with minimum release during the second week. Meanwhile, the release profile of the 5% formulation seems to stabilize at about four days after releasing close to 30%. This suggests that **pilocarpine is present in two forms**--loosely and tightly bound. The loose fraction is associated with the fast release segment of the profile. Thus, for future studies, attempts should be made to determine the point at which the tightly bound pilocarpine is released. This may take place as the gel-former degrades. An attempt to interrupt the *in vitro* experiment at one week to determine percent of residual drug in the gel was not fruitful.

For the **5% and 10% ganciclovir (GC as the sodium salt)** in 50 mg formulation, the average cumulative release profiles (based on duplicate runs) are depicted in Figures 2a and 2b and provided numerically in Table I. The profile of the 10% formulation shows a release of about 64% at one week, followed by a steady, but very slow, release for an additional 13 days. For the 5% formulation, about 78% was released during the first week, and less than 10% continued to release during the succeeding 13 days. As proposed in the pilocarpine case, GC may be present in loosely and tightly bound forms in the gel. In future studies, one of the *in vitro* experiments will be interrupted to determine residual drug concentration at the 1-week period.

The available release data (based on duplicate runs) of **5% and 8% cyclosporin A (CS),** 50 mg formulations, are given in Table I and illustrated in Figures 3a and 3b. The results reflect exceptionally slow, but continuous, release profiles for both formulations. The 5% formulation releases about 14% and less than 20% at 17 and 31 days, respectively. Meanwhile, the 8% formulation releases about 8% and 10% at 17 and 26 days, respectively. This extraordinarily slow release profile of CS may be associated with the drug's hydrophobic nature, low water solubility, and affinity to the polymeric vehicle. Analysis of residual drug in the gel at one week was deemed impractical during the allocated time slot. And most of our efforts were directed towards **refining the HPLC analytical protocol** to procure more reproducible data than those reported in the Phase II application.

Using 50/50 mixtures of free **naproxen (NP)** and its sodium salt, at 5% (in duplicate) and 10% (in triplicate) concentrations in 50 mg formulation to conduct the *in vitro* release studies, led to the release profiles shown in Figures 4a and 4b, and expressed numerically I Table I. The results indicate that for the 10% formulation, about 78% of the drug is released at 7 days, followed by very slow, but continued, release during the succeeding 20 days. For the 5% formulation, about 67% of the drug was released in 7 days, but continued to release very slowly for an additional period of 15 days. Similar to PC and GC, NP appears to exist in loosely and tightly bound forms. However, the loosely bound form appears to be the major fraction. In NP, the fast release is expected to be primarily due to the water-soluble salt. Therefore, in future studies, the less water-soluble free acid will be used. Due to time constraints and need to refine the HPLC analytical protocol to obtain more reproducible data than those reported in the Phase II application, the residual drug concentration in the gel at one week was not determined.

D.2.4. Comparative Analysis of the *In Vitro* Release Profiles--Available data suggest that (1) the overall release rates of the four drugs can be compared in the following order, NP $\geq$ GC > PC >> CS; (2) drug water solubility is a major factor in determining the release profile; (3) drug loading can have an effect on the release profile; (4) the water-soluble drug may exist in loosely and tightly bound forms in the gel; and (5) high drug concentration may affect the rate of gel absorption by hydrolysis, especially with basic drugs.

D.3. Animal Studies--This section deals with the (1) highlights of the revised animal protocol; (2) pilot studies A and B; (3) main study A; (4) main study B; (5) histological studies; and (6) collective analysis of the results of the animal studies.

D.3.1. Highlights of the Revised Animal Protocol--The **original** protocol was **revised and expanded** because of the (1) diverse physicochemical properties and therapeutic efficacy of the four drugs used in the study and uncertainty about their interaction with the gel-former; (2) unavailability of tissue compatibility data for the gel-former; (3) first-time use of one or more of these drugs in the designated administration sites; and (4) need to use drug loading that can lead to detectable biological events and drug concentrations which can be monitored within the detectable limits of available analytical procedures.

The animal model for this project was the **New Zealand white rabbit**. The study was divided into four sections, a **two-part pilot study** (Figure 5) and a **two-part main study** (Figure 6). The **first pilot study** was planned to assess the **biocompatibility** at 1 and 21 days of two injectable gel-formers administered via subconjunctival and intravitreal injections. For this pilot study, four rabbits were required. The 21-day compatibility data were to be used as controls in assessing active formulations at three weeks. One day acceptable compatibility data permitted the initiation of the second pilot study. **The second pilot study** was intended to determine the most suitable **administration site**, subconjunctival or intravitreal, and to select an appropriate gel-former. Eight rabbits were needed for this segment of the study. In both pilots, the eyes were examined by the attending veterinarian and/or a veterinary ophthalmologist. The eyes from

the first pilot study were saved for potential histological evaluation. The eyes from the second pilot study were saved for potential evaluation of drug content. After the gel-former biocompatibility was verified and the gel-former type and administration site were selected in the pilot studies, the main study was initiated.

The **main study** was also divided into **two parts**. The **first part** was designed to determine the **appropriate dosage** of the four selected drugs. Eight rabbits were required for this part of the study. Each rabbit received an injection of a gel-former containing either a high or a low dose of one of the four drugs. During the study, the animals were observed for any adverse effects of the drug at high concentration levels. At the conclusion of this part of the study, the eyes were collected and analyzed for residual drug content. Once the drug dosage was determined, the **second part** of the main study commenced using thirty-six rabbits. In this segment, each rabbit received an injection of gel-former containing one of the four drugs at a dose determined in the first part of the study. Rabbits were euthanized at time periods outlined on the accompanying flow chart (Figure 6), and eyes were collected and analyzed for residual drug content in the retrieved gel. Three rabbits per formulation per time period were required for this study and subsequent determination of **the *in vivo* release profile** of the four drugs. As in the pilot study of this project, the rabbits were regularly examined as in both parts of the main study by the attending veterinarian and/or a veterinary ophthalmologist.

Table I and Figures 1a and b, 2a and b, 3a and b, and 4a and b follow on pages 20, 21, 22.
Figures 5 and 6 depicting the revised animal protocols are shown on pages 23, 24.

Table I. ***In Vitro*** **Release Profile Data: Percent Drug Released Over Time.**

Drug	% Drug in Gel	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17
Pilocarpine	10	--	36.0	40.5	43.8	45.0	45.9	46.2	46.3	46.3	--	--	--
Pilocarpine	5	17.0	29.6	33.3	34.2	34.5	34.7	34.9	--	35.6	35.7	35.8	--
Naproxen	10	--	46.3	60.3	71.7	76.3	78.0	78.4	78.9	79.3	79.6	79.8	80.0
Naproxen	5	19.3	48.6	57.7	62.8	63.3	66.7	67.3	67.9	68.1	68.4	68.6	68.8
Ganciclovir	10	--	38.0	50.9	59.3	61.3	62.6	63.6	63.9	65.1	65.4	65.8	66.1
Ganciclovir	5	--	41.9	58.1	68.3	73.7	76.9	78.2	80.4	81.6	82.8	83.7	85.3
Cyclosporin A	8	0	0	0.5	--	1.4	2.3	2.6	3.6	3.8	5.2	7.3	8.3
Cyclosporin A	5	0.2	1.1	2.6	2.6	4.1	4.8	5.4	6.4	7.3	--	11.0	13.8

Note: The values above were obtained from the average of at least two identical release experiments.

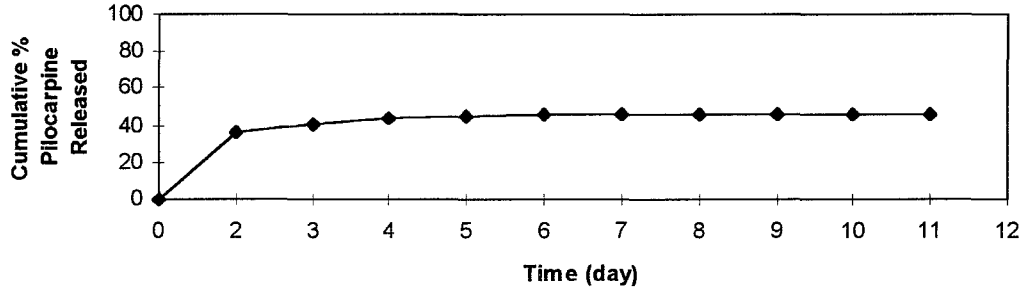


Figure 1a. Cumulative *In Vitro* Release Profile of 10% Pilocarpine in GF55 Gel.

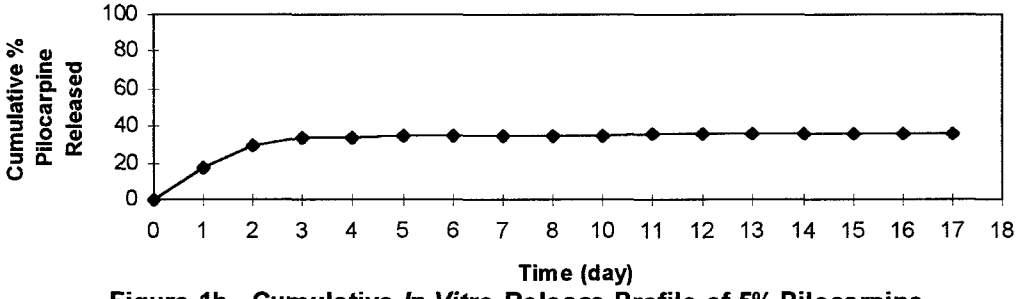


Figure 1b. Cumulative *In Vitro* Release Profile of 5% Pilocarpine in GF55 Gel.

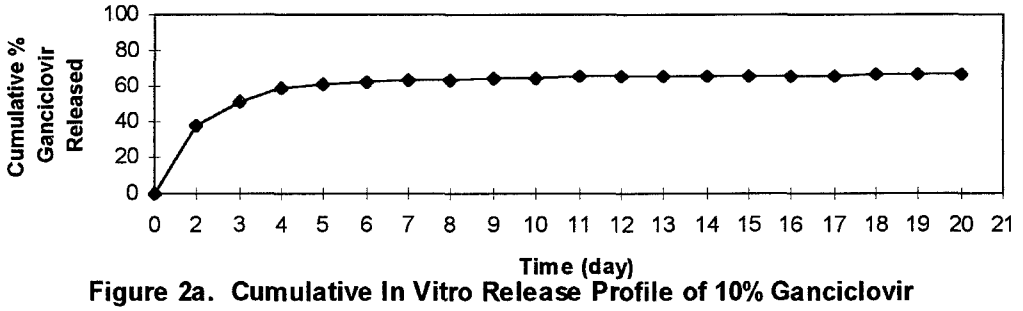


Figure 2a. Cumulative In Vitro Release Profile of 10% Ganciclovir in GF55 Gel.

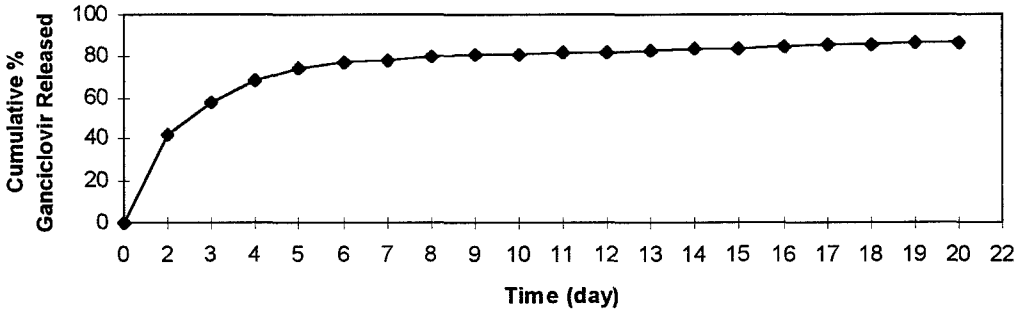


Figure 2b. Cumulative *In Vitro* Release Profile of 5% Ganciclovir in GF55 Gel.

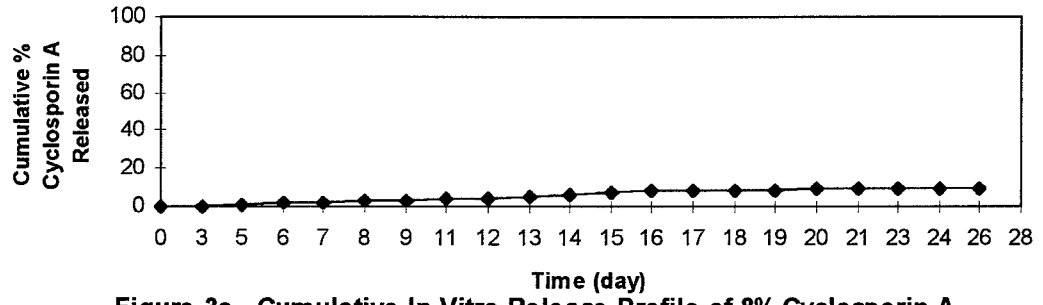


Figure 3a. Cumulative In Vitro Release Profile of 8% Cyclosporin A in GF55 Gel.

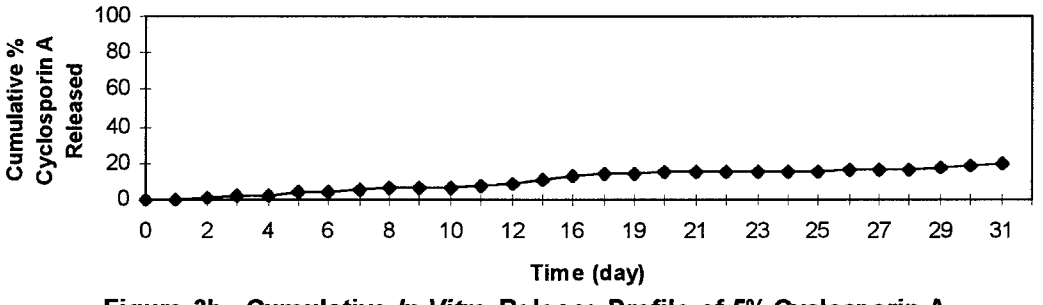


Figure 3b. Cumulative *In Vitro* Release Profile of 5% Cyclosporin A in GF55 Gel.

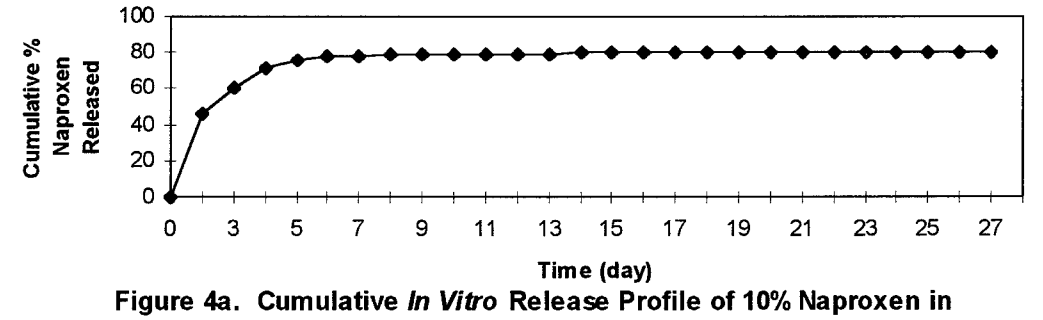


Figure 4a. Cumulative *In Vitro* Release Profile of 10% Naproxen in GF55 Gel.

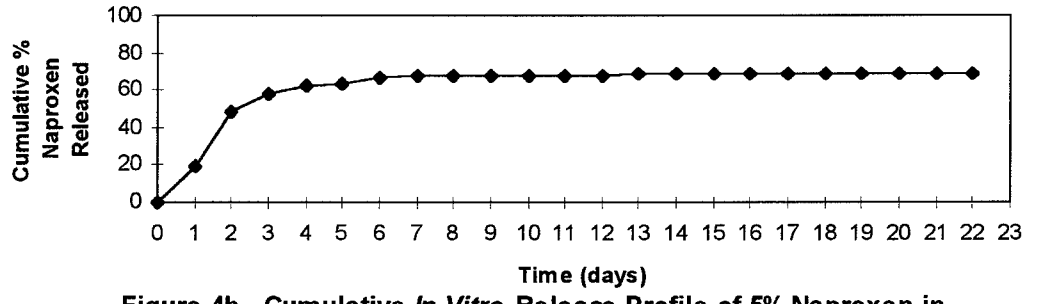
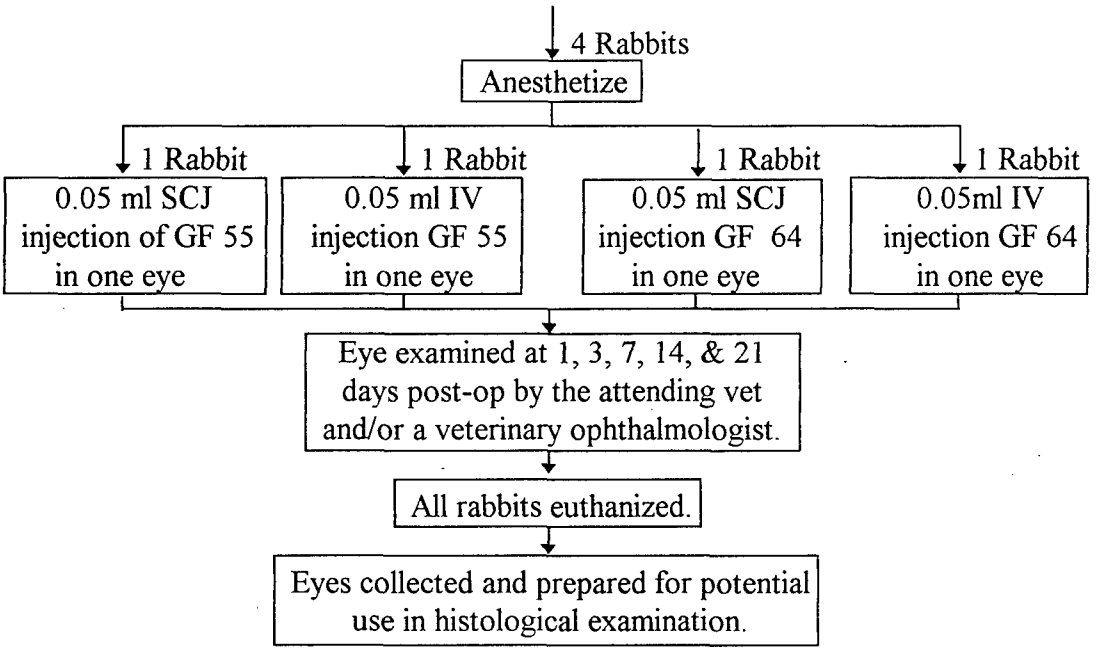
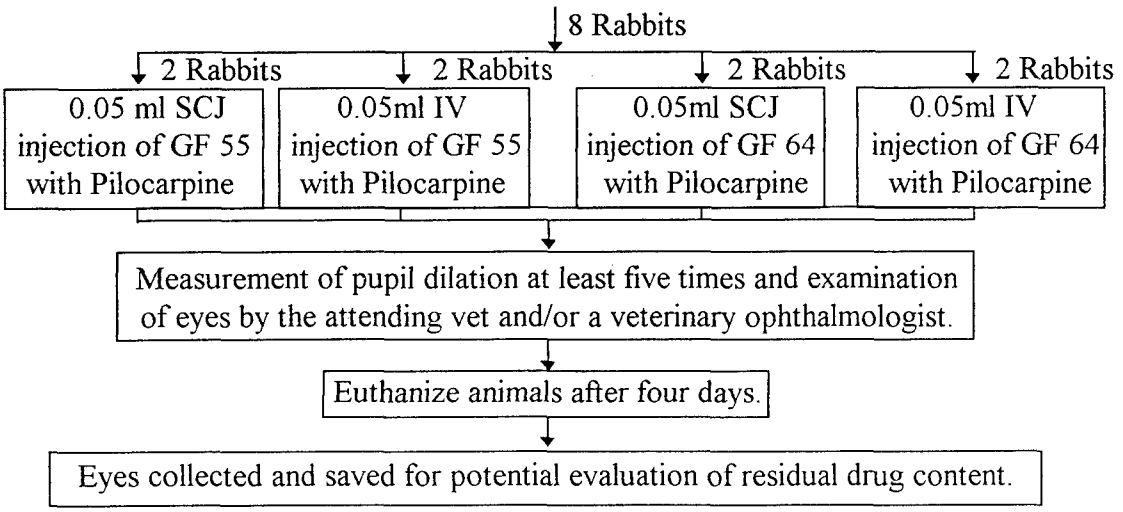


Figure 4b. Cumulative *In Vitro* Release Profile of 5% Naproxen in GF55 Gel.

Pilot Study A: Gel Compatibility



Pilot Study B: Administration Site and Gel Selection*



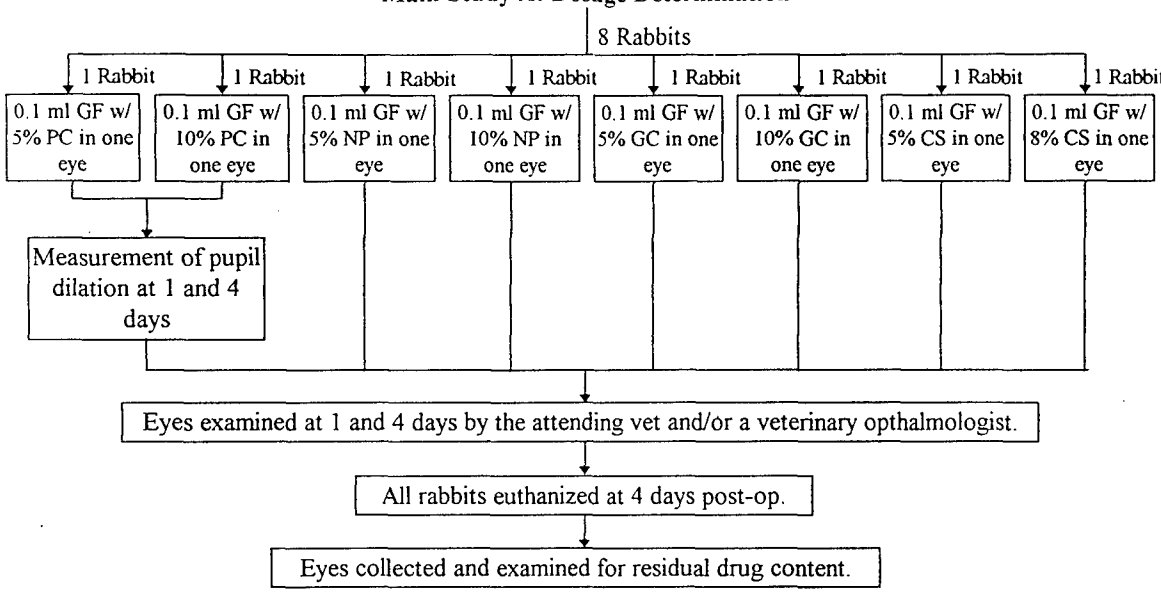
*Pilot Study B is to be started on the second day of Pilot Study A, barring any unexpected problems with gel compatibility.

SCJ = Subconjunctival

IV = Intravitreal

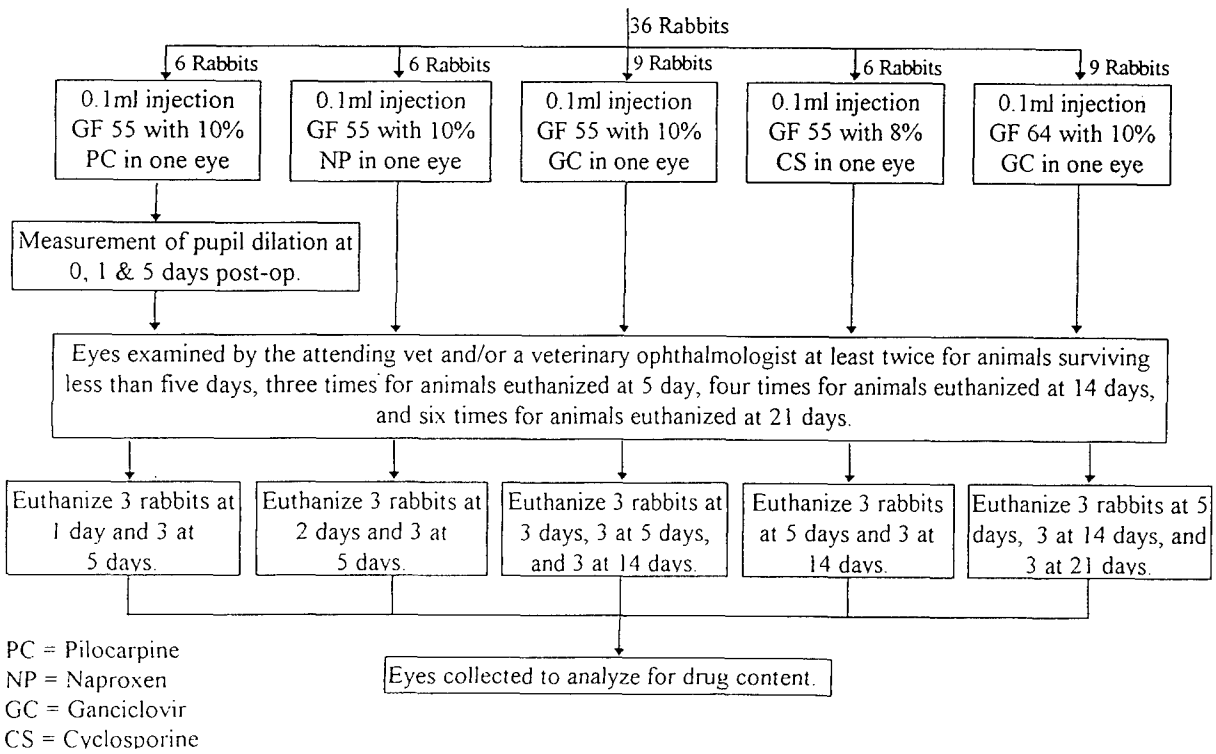
Figure 5

Main Study A: Dosage Determination



Note: From the pilot studies it was determined that GF=GF 55, and the formulation will be added intravitreally.

Main Study B: Injectable Absorbable Ocular Inserts for Controlled Drug Delivery



PC = Pilocarpine
NP = Naproxen
GC = Ganciclovir
CS = Cyclosporine

Figure 6

D.3.2. Pilot Animal Studies--The first component of the pilot studies (**Pilot A**) was intended to (1) determine the **feasibility of injection 0.05 to 0.1 ml** of GF-55 using a 27 gauge needle into the vitreous humor and conjunctiva of the rabbit eye; and (2) assess any **gross tissue reaction** to the gel-former, at least in the vitreous humor. To this end, it was found that (1) 0.05 ml of the formulation can be easily introduced into the vitreous humor and conjunctiva sites; (2) introducing the 0.1 ml of the gel-former is more difficult to implement; and (3) as per gross visual observations of the treated rabbits over a three-week period, the presence of GF-55 in the ocular sites is not associated with discernible tissue reactions at 7-10 days post-administration. To verify the latter observation, the rabbits were euthanized at three weeks for conducting pertinent histological studies. The second component of the pilot study (**Pilot B**) was intended, primarily, to determine if the **subconjunctival** administration, which is easier to implement, is a viable alternative for the more delicate **intravitreal** administration. A second objective of Pilot B was later adopted and deals with identification of the **minimum injectable dose of a model drug** (such as pilocarpine) that can be used to determine differences in the drug pharmacodynamics at both sites. To this end, 0.05 ml of a 0.236% pilocarpine formulation was injected in the vitreous cavity and conjunctiva of two separate sets of four rabbits. Over a period of three days, less than 40% of the rabbits showed no significant response to the released drug and no definite site dependence. Thus, the experiment was repeated using a three-fold increase in the drug concentration (0.05 ml formulation containing 0.7% pilocarpine). Such an increase in drug dose led to practically no change in response. Meanwhile, there was no discernible difference in terms of site performance. Therefore, 0.1 ml of GF-55 containing 5% pilocarpine was injected intravitreally, and a strong response was achieved. Subsequently, the 5% drug concentration was selected as the low level dose for the four drugs to be examined in subsequent segments of Phase I.

D.3.3. Main Study A: Dose Determination--Considering the discussion in Section D.2.4., the original flow charts were revised as shown in Figure 6. On the basis of Pilot B results, comparable performance of SCJ (subconjunctival) and VC (vitreous cavity) sites, it was decided to use the more invasive VC site for the main study. This was consistent with potential need to easily retrieve the gel at the conclusion of the study, and gel-former GF-55 was selected for the study. Results of this component of the main study indicated that for PC, NP, and GC, it was feasible to use up to 10% drug in the formulations for intravitreal injections. Upon using the low concentration (5%) of CS, reaction to the drug during the first few days following treatment was much more discernible than those of PC, NP, and GC. Accordingly, the upper limit for the CS formulation was set at 8% concentration. And results indicate that 8% concentration can be tolerated by the rabbit eye. Accordingly, the *in vivo* release study in the Main Study B was based on **10% formulation for PC, NP, GC, and on 8% loading in the CS** formulation.

Using a number of the intravitreally treated globes, a preliminary study was conducted to determine the proper protocol for determining the drug concentration in different eye compartments. Results of these experiments indicated that for eyes treated with 5% and 10% drug formulation, determination of the drug concentration in the vitreous fluid or homogenized tissue is impractical using extraction followed by HPLC. Therefore, it was decided to explore retrieving residual gel components retained in the aqueous humor at specific periods of time and determine their drug concentration. Although the retrieval approach was found to be tentatively feasible, the mechanics of recovery were found to be far from optimal. And preliminary retrieval results (Table II) do illustrate that only a small fraction of the residual gel has the physical integrity and size suited for mechanical retrieval. Nevertheless, this approach was considered more feasible than processing the vitreous humor or the entire eye tissues. Accordingly, the retrieval approach was adopted and the protocol was modified to maximize the amount of retrieved gel and facilitate the analysis for the remaining drug. Thus, an optimum protocol was adopted as in Section D..3.4. which is based on (1) refining the retrieval procedures to minimize excessive fragmentation of the gel during processing as shown in a typical case of CS formulation (Section D.3.4.); (2) using high loading of drug, namely, 8-10% formulation; and (3) maintaining the vitreous humor as the experimental site for administration.

Table II. Main *In Vivo* Study A: Preliminary Data on Gel Retrieval from the Vitreous Humor

Rabbit	Drug (loading)	Time Point (days)	Gel Recoverable dry wt. (mg)	Gel Implanted initial wt (mg)	Average Percent Recoverable Gel
S14	Pilocarpine (5%)	4	3.1*	100	3.1*
S15	Naproxen (5%)	4	0	100	0
S16	Cyclosporin A (5%)	4	0	100	0
S17	Ganciclovir (5%)	4	6.7	80	8.4
S18	Cyclosporin A (8%)	4	21.0	70	30.0
S19	Pilocarpine (10%)	4	24.7	80	30.9
S20	Ganciclovir (10%)	4	0	100	0
S21	Naproxen (10%)	4	19.0	100	19.0

*S14: entire vitreous was not available for collection of gel

Using the aforementioned retrieval protocol discussed above **prior to its modification**, gel formulations having 5% to 10% concentration of the four drugs were isolated and analyzed for remaining drug levels at four weeks. The respective results, which are shown in Table III, indicate that (1) drug loading has hardly any effect on total release at four weeks; and (2) with the exception of CS, all drugs are fully released at four weeks regardless of loading. This, the difficulty in analyzing very small drug concentrations, and the acceptable site reaction to 8% CS and 10% NP, PC, and GC, **substantiated our selection** of these concentrations for Segment B of *the in vivo* study.

Table III. Main *In Vivo* Study A: Data on Optimum Drug Loading Determination

Rabbit	Drug Released	Time Point(day)	% Drug Released
S19	Pilocarpine (10%)	4	99.69
S14	Pilocarpine (5%)	4	99.96
S17	Ganciclovir (5%)	4	99.98
S18	Cyclosporin A (8%)	4	30.06
S21	Naproxen (10%)	4	99.99

Note: These results were used to determine appropriate drug levels to use in Main Study B

D.3.4. Main Study B: Drug Release by Intravitreally Injected Formulation--Taking into account our experience in Main Study A, it was decided to (1) use 10% as the drug loading for PC, NP, and GC; (2) limit the loading in formulations of CS to 8%; (3) prepare all formulations with GF-55; and, hence, (4) the additional set of animals planned initially for CS in a second gel was revised to use 10% GC formulation in GF-64. (See Figure 6.)

Toward optimizing the gel **retrieval process** and its use to determine concentration of drug remaining in the gel at different periods, the following **protocol was developed**: Individual sets of rabbits treated intravitreally with a specific formulation are euthanized at designated periods of time. The globes are then removed and kept frozen until ready to determine residual drug in remaining gel fragments (to be retrieved from vitreous humor). For each individual formulation at a given period, a number of frozen globes (eyeballs) are thawed, incised to remove residual fragments of the vitreous humor, and then refrozen (for

any future processing). The removed gel particles were dried under reduced pressure to a constant weight to determine their total weight. The dried gel particles were then dissolved in acetonitrile. After adding an equal volume of water to precipitate the polymer, the mixture was centrifuged. The supernatant liquid was then analyzed by HPLC to determine drug concentration. In a first test of this protocol to verify its appropriateness, two sets of globes treated with an 8% CS formulation were used as typical cases. And results associated with the *in vitro* release profile of CS were recorded. Pertinent data of the extraction and analysis are shown in Table IV.

Table IV. Analysis of Typical Gels Recovered from Rabbit Globes--Results for 8% Cyclosporin A Formulation

Rabbit Number	Study Period (Days)	% CS in Gel*	
		Individual Values	Average Values
S-43	5	57	
S-44	5	56	54.5
S-45	5	51	
S-46	14	72	
S-47	14	66	61.3
S-48	14	46	

* The average percentages of recovered mass for the 5- and 14-day animals were about 50 and 40% of the original mass, respectively. The calculated percentages were based on a 100% recovery of CS from the gel.

The data in Table IV indicate that CS is released slowly from the gel formulation. However, the *in vivo* release profile appears to be faster than that determined in the *in vitro* release study. This may be attributed to the presence of lipophilic species in the vitreous humor that increase the drug solubility and diffusion to the biological environment. It is also interesting to note that the average percent of drug remaining in the gel at 14 days was higher than expected. However, this may be due to the fact that the gel mass decreases substantially, as the matrix absorbs due to biodegradation, between day 5 and day 14, which raises the apparent drug loading. This, in turn, may reflect the effect of the drug in catalyzing the hydrolysis and absorption of the polyester component of the gel.

The process described above for CS was later used in gel retrieval of PC, NP, and GC gel formulations at different periods as shown in Table V. Comparative analysis of the results in Table V of the four drugs and the *in vitro* release data in Table I indicate, in general terms, that (1) the recoverable mass of the gel is expected to be less than actual mass since microgels and swollen gel particles having a refractive index similar to that of the vitreous humor are difficult to recognize, and hence, retrieved; (2) the percent of recoverable gel varies from about 7 to 55; (3) the fraction of recoverable gel decreases with residence time in the vitreous humor; (4) high drug solubility in water and fast release rates can result in lower values of recoverable gel; (5) high drug basicity, especially in low water solutility drugs (e.g., cyclosporin A), can be related to low recoverable fractions at advanced periods because of their effect in catalyzing the polymer degradation; and (6) the hydrophobicity and the gel-forming carrier can influence its degradation leading to substantial increases in the fraction of recoverable gel--this is well illustrated in the case of the GS formulations.

More specific comments on the individual drugs can be noted as follows: (1) The limited effect of residence time on the amount of recoverable gel containing naproxen may be related to having a 50/50 mixture of the water soluble sodium salt and the practically insoluble free acid. Thus, the release of this

mixture appears to occur in two steps, fast and slow, due to the salt and free acid, respectively. (2) A high drug loading (Table I) of the basic CS may accelerate its drug release through accelerating the degradation and fragmentation of the gel matrix. (3) Pilocarpine formulation releases the drug in fast and slow steps, which may be related to having the drug in two distinctly different forms in terms of water solubility.

Table V. Main *In Vivo* Study B: Percent Recoverable Gel at Various Time Points

Rabbit	Drug *	Time Point (days)	Gel Recoverable dry wt. (mg)	Percent Wt Loss	Average Percent Recoverable Gel
S22	Pilocarpine	1	42.2	57.8	-
S23	Pilocarpine	1	58.5	41.5	**54.53**
S31	Pilocarpine	1	62.9	37.1	-
S24	Pilocarpine	5	49.8	50.2	-
S25	Pilocarpine	5	49.8	50.2	**50.23**
S26	Pilocarpine	5	51.1	48.9	-
S27	Naproxen	2	37.2	62.8	-
S28	Naproxen	2	40.1	59.9	**41.0**
S29	Naproxen	2	45.7	54.3	-
S30	Naproxen	5	39.2	60.8	-
S32	Naproxen	5	39.0	61.0	**39.67**
S33	Naproxen	5	40.8	59.2	-
S43	Cyclosporin A	5	50.1	49.9	-
S44	Cyclosporin A	5	49.5	50.5	**50.8**
S45	Cyclosporin A	5	52.8	47.2	-
S46	Cyclosporin A	14	36.1	63.9	-
S47	Cyclosporin A	14	39.3	60.7	**40.8**
S48	Cyclosporin A	14	47.1	52.9	-
S34	Ganciclovir	3	5.5	94.5	-
S35	Ganciclovir	3	26.8	73.2	**16.47**
S36	Ganciclovir	3	17.1	82.9	-
S37	Ganciclovir	5	10.0	90.0	-
S38	Ganciclovir	5	6.8	93.2	**8.4**
S39	Ganciclovir	5	8.4	91.6	-
S49 ψ	Ganciclovir	5	14.9	85.1	-
S50 ψ	Ganciclovir	5	14.3	85.7	**15.1**
S51 ψ	Ganciclovir	5	16.0	84.0	-
S40	Ganciclovir	14	5.2	94.8	-
S41	Ganciclovir	14	3.2	96.8	**3.2**
S42	Ganciclovir	14	1.4	98.6	-
S52 ψ	Ganciclovir	14	10.4	89.6	-
S53 ψ	Ganciclovir	14	35.7	64.3	**19.1**
S54 ψ	Ganciclovir	14	11.1	88.9	-
S55 ψ	Ganciclovir	21	9.0	91.0	-
S56 ψ	Ganciclovir	21	8.7	91.3	**6.77**
S57 ψ	Ganciclovir	21	2.6	97.4	-

*All drugs were loaded at 10% except for cyclosporin A which was loaded at 8%. ψ Denotes formulations based on GF-64; all others are based on GF-55.

Following the protocol tested with the 8% CS formulation, the ***in vivo* release data for the remaining formulations** were obtained by analyzing the respective fragments of retrieved gel masses from globes at different time periods, namely, 1 to 14 weeks. The **results, as summarized in Table VI**, can be summarized as follows:

1. **For pilocarpine**, the apparent release profile suggests that most of the drug is released in the first day. This suggests an exceptionally faster rate than observed in the *in vitro* study. More importantly, the two-step release profile noted under the *in vitro* conditions could not be detected in the *in vivo* release. This may be attributed to a substantial increase in solubility of PC hydrochloride or the free base in the vitreous humor in the presence of surfactant biomolecules.

2. In the case of **ganciclovir**, in spite of the noted fast release profile, the drug was still present in the retrieved gel **at 21 days**. The effect of gel hydrophobicity on the release profile, which was observed in the *in vitro* release data, appears to be reversed in the *in vivo* case. Once again, this may be related to the presence of surfactant biomolecules which alter the drug solubility. This may also suggest that in future *in vitro* release studies one may add a phospholipid to the phosphate buffered solution for better simulating the biological environment.

3. The *in vivo* release profile of **cyclosporin A** is 3 to 4 times that recorded in the *in vitro* study. Here, the effect of surfactant biomolecules on the drug solubility and release is much more pronounced as compared with PC and GC. These results show clearly that the gel-former systems **are well suited for the controlled delivery of CS** and, similarly, hydrophobic polypeptides. The lower release at 14 days, as compared with that at 5 days, was explained earlier as being primarily related to biodegradation of the polyester gel matrix about the drug leading to an "apparent" high concentration in the remaining mass.

4. The *in vivo* release results of **naproxen** are in concert with the note made earlier regarding the *in vitro* release where a two-step profile was proposed. Thus, at 2 days, the soluble sodium salt of the drug is released. This is followed by the less soluble free acid. In any event, the overall *in vivo* release profile is faster than that recorded in the *in vitro* situation. Meanwhile, through the proper adjustment of the drug composition as well as molecular weight and hydrophobicity of the gel-former, it may be possible to modulate the release profile of naproxen as well.

Table VI. Main *In Vivo* Study B: Drug Release Data

Drug Released*	Time Point (day)	Number of Studies Averaged	Average % Drug Remaining from Recoverable Gel	Average % Drug Released from Recoverable Gel
Pilocarpine	1	1	7.78	92.22
Pilocarpine	5	5	3.17	96.83
Ganciclovir	3	2	0.24	99.76
Ganciclovir	5	2	7.11	92.88
Ganciclovir ψ	5	3	2.33	97.67

Table VI Cont'd.

Drug Released*	Time Point (day)	Number of Studies Averaged	Average % Drug Remaining from Recoverable Gel	Average % Drug Released from Recoverable Gel
Ganciclovir	14	2	8.06	91.94
Ganciclovir ψ	14	3	2.01	97.99
Ganciclovir ψ	21	3	0.41	99.59
Cyclosporin A	5	3	54.5	44.88
Cyclosporin A	14	3	61.3	38.02
Naproxen	2	2	40.73	59.27
Naproxen	5	5	2.05	97.95

Note: Cyclosporin A is not normalized with an extraction efficiency value. *All drugs were loaded at 10% except for cyclosporin A which was loaded at 8%. ψ Denotes gel GF-64 compositions; all others are based on gel GF-55.

D.3.5. Histological Evaluation--This section entails (1) the general procedure for preparing histological specimens; (2) description of examined sets of specimens and overall histological evaluation of each set; and (3) overall assessment of the results.

a. Preparation of histological specimens--Globes were fixed in Davidson's solution. In all cases, two sections through a mild transversely sectioned globe were examined unless otherwise indicated. Sections were processed routinely and stained with hematoxylin and eosin.

b. Description of examined sets of specimens

Set I.--Description

I.D.	Source	Administration Route*	Dose, 50 ml	Study Period
S2 OD	Pilot A	IV	GF-55	23 days
S2 OS	Pilot A	None	Control	23 days
S5 OD	Pilot A	SCJ	GF-55	23 days
S5 OS	Pilot A	--	Sham	23 days
S8 OD	Pilot B	IV	GF-55 + 0.236%, PC	34 days

* IV = intravitreal SCJ = subconjunctival

Comparative evaluation--Subconjunctival injection of the polymer appears to elicit a focal pyogranulomatous to lymphogranulomatous reaction with little involvement of adjacent tissues or the globe itself. The globe in the first intravitreal injection (S2 OD) does not differ histologically from the control or sham injected globes. The second intravitreal injected globe (S8 OD) has an area of lens rupture. Whether

this is due to the injection procedure or contact with the polymer is unknown at this time. It should also be noted that the polymer was not visible in routine histologic section of paraffin embedded tissue.

Set II.--Description

I.D.	Source	Administration Route*	Dose, 50 ml	Study Period
S7 OD	Pilot B	SCJ	GF-55 + 0.236% PC	56 days
S7 OS	Pilot B	SCJ	GF-55 + 0.71% PC	49 days
S9 OD	Pilot B	IV	GF-55 + 0.236% PC	56 days
S9 OS	Pilot B	IV	GF-55 + 0.71% PC	49 days

* IV = intravitreal SCJ = subconjunctival

Comparative evaluation--Subconjunctival injection of the polymer in **S7 OD** appears to elicit a more severe reaction in the connective tissue than the polymer injected in **S7 OS**. The reaction in both cases, however, is focal and does not involve the globe or intraocular tissues. The globe in **S9 OS** has a reaction to the intravitreal injected polymer that is severe enough to cause involvement of the retina and choroid. In contrast, the second intravitreal injected globe **(S9 OD)** has only mild reaction to the injected polymer.

Set III.--Description

I.D.	Source	Administration Route*	Dose, 50 ml	Study Period
S7 OS	Pilot B	SCJ	GF-55 + 0.71% PC	49 days
S10 OD	Pilot B	SCJ	GF-55 + 0.71% PC	72 days
S11 OD	Pilot B	SCJ	GF-64 + 0.235% PC	79 days
S11 OS	Pilot B	SCJ	GF-64 + 0.71% PC	72 days
S12 OD	Pilot B	IV	GF-64 + 0.236% PC	84 days
S12 OS	Pilot B	IV	GF-64 + 0.71% PC	77 days

* IV = intravitreal SCJ = subconjunctival

Comparative evaluation--No inflammatory infiltrate was seen either grossly or histologically in the samples taken from **S11 OD** and **S12 OD**. This may be due to sectioning of the samples or to the fact that the compounds injected failed to elicit an inflammatory response. With the exception of S11 OS, the other samples had infiltrates similar to those seen in previously reported cases. The reaction in S11 OS could be interpreted in several ways--a reaction to pre-existing cataract, inadvertently intravitreal injection, or intraocular inflammation unrelated to the cataract or an injected polymer.

c. Overall assessment of the results--The data generally indicate that (1) subconjunctival injection of the placebo gel-former (50 µl) elicits a focal pyogranulomatous to lymphogranulomatous reaction with little involvement of adjacent tissue or globe itself; and (2) globes treated **with gel-former do not differ histologically from the control** (eye puncture only) or sham injected globes. This justified further our earlier selection of the vitreous over the subconjunctival site.

D.3.6. Collective Analysis of the Animal Study--Available *in vivo* release data and histological evaluation results indicate that (1) GF-55 is suitable for subconjunctival and **intravitreal injection in 50 to 100** µl volumes; (2) the gel-former **does not elicit adverse reaction** at and about the injection site beyond the initial traumatic period; (3) during the absorption of the gel-former, and well **beyond its absorption** (at or before 6 weeks), it **does not elicit adverse tissue reaction** in the eye; (4) although **5% to 10%** drug loadings (with the exception of CS, where the maximum loading is **8%) can be tolerated** by the eye, much lower concentrations will be required for clinical efficacy; (6) drug concentration, hydrophilicity, solubility, and basicity (or acidity) do affect the release and absorption profile of the gel-former; and (7) gel-forming formulations are likely to be suitable **vehicles for several other ophthalmic drugs** and may be administered both **intraocularly and topically**.

E. CONCLUSIONS AND RECOMMENDATIONS

E.1. Conclusions

1. Gel-former-based formulations with 5%-10% drug loading could be used as injectable, intraocular controlled delivery systems for intravitreal and subconjunctival administration in 50 to 100 µl aliquots without eliciting discernible adverse tissue reaction to the four drug formulations or their placebo.

2. The hydrophobicity, molecular weight, and hydrolytic stability of the gel-forming gel-formers are the most important parameters for controlling the release profile, particularly for water-soluble drugs such as naproxen sodium, ganciclovir hydrochloride, and ganciclovir sodium.

3. For drugs with very limited water solubility, such as the free acid of naproxen and cyclosporin, the major factors in determining their release profiles are (a) the extent of water solubility; (b) molecular weight; (c) their catalytic effect on the hydrolysis of the polyester components of the absorbable matrix; and (d) presence of lipophilic components in the liquid environment about the gel.

4. Gel-forming formulations are likely to be safe vehicles for other drugs and are well suited for both intraocular and topical ocular administrations.

5. The continuous flow-cell system designed and employed for monitoring the *in vitro* release profile could be used for accurate, comparative study of the *in vitro* controlled release of different drug formulations to timely modulate the formulations for the *in vivo* study.

E.2. Recommendations

1. To pursue, as part of Phase II, development studies, scale-up studies, and safety studies on ganciclovir as an injectable intravitreal formulation using a more hydrophobic, higher molecular gel-former than that used in Phase I.

2. To select, as part of Phase II, a more potent alternative to pilocarpine (e.g., dorzolamide or the phenyl derivative of prostaglandin, F2α), and pursue development, scale-up, and safety studies as an injectable subconjunctival or topical formulation using a higher molecular weight, more hydrophobic gel-former than those used in Phase I.

3. To explore further (in Phase II), the development of cyclosporin A and naproxen (as the free acid) as injectable, subconjunctival, or topical formulations for established indications.

4. To explore (in Phase II) the use of the gel-forming system for the controlled release of cidofevir (intravitreally) as a potentially more potent substitute for ganciclovir.

5. To assess (in Phase II) extending the gel-former technology to galardin for corneal ulcer treatment and 5-fluorocil as an antiproliferative agent.

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APPENDIX A

List of personnel at Poly-Med, Inc., receiving pay for this project:

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APPENDIX B

Publication

Shalaby, S.W., Structure Modulation and Evaluation of Absorbable/Biodegradable Materials, IUPAC Symposium on Molecular Architecture for Degradable Polymers, Stockholm, Sweden, June 10-13, 1997.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
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